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According to the Pre Publication Rules, every patent application received by the United States Patent and Trademark Office after November 29, 2000 will be pre-published at eighteen months from the effective filing date. When the application is published the contents, including the sequences, will become prior art.

Two new databases have been created to hold the pre-published sequences:
Published_Applications_NA contains nucleic acid sequences; the search results will have the extension **.rnpb**.
Published_Applications_AA contains amino acid sequences; the search results will have the extension **.rapb**.

Each pre-published application is given a unique Publication Number. An example of a Publication Number is US20021234567A1. The "US" indicates the application was a U.S. application. The first 4 digits show the calendar year the application was published. The next 7 digits represent when the application was published. This 7-digit number starts at zero at the beginning of each calendar year. Each application published is given the next number in order. The "A" indicates a utility patent application and the "1" shows that this was the first time the application had been published. If the applicants submit changes to the application, they may requests that the changed application be published again. In such instances, the "1" at the end of the number would be replaced by a "2".

Sequences in the PGPub database are public information; it is permissible to leave these results in the case.

Dear Examiner,
The attached search was run with the most recently released version of Compugen's search software, GenCore 5. With this update, several changes have occurred in the results of FrameSearches (protein query sequence vs nucleic acid databases or nucleic acid query sequence vs protein databases).

In reference to FrameSearches:

- The output format has been improved so that it more closely resembles the format for standard search output.
- Calculation of Percent Similarity has been changed for FrameSearches. The new method of calculation is more similar to the method used in NCBI's BLAST algorithm. The same results are found in the same order using GenCore 5 and the previous version of GenCore, but Percent Similarities are lower in GenCore 5 results.

  - The formula for % similarity calculation is:

$$100 * \frac{\text{matches + conservative substitutions}}{\text{alignment\_length}}$$

    where "matches" is the number of identical matches and "conservative substitutions" is the number of non-identical positive matches.

  - GenCore 4.5 considers the match Thr vs GCT (Ala) to be a similarity since BLOSUM62 gives score of 0 to this match. It is marked by ':::' in the alignment:

```
Qy     46 AspSerThrAspAla.Met..Gly 52
          ||||||:::  |||  :::  |||
Db    605 GATTCCGCTGCTGCTAATTTTGGC 628
```

    GenCore 5 requires a positive score to consider a non-identical match a similarity, therefore the same 'match' is not emphasized in the new alignment:

```
Qy     46 AspSerThrAspAla.Met..Gly 52
          ||||||      |||  :::  |||
Db    605 GATTCCGCTGCTGCTAATTTTGGC 628
```

If you have any questions, please feel free to contact one of the searchers in Biotech/Chem Library.

STIC-ILL

NO

42628

From: Portner, Ginny
Sent: Thursday, January 02, 2003 4:14 PM
To: STIC-ILL
Subject: FW: 09/727,892

Importance: High

Journal of Diary Science, Oct. 1998, Vol. 81(10), pages 2771-2778. Bower, CK et al

9812283

Ginny Portner
CM1, Art Unit 1645
Room 7e13
Mail box 7e12
(703) 308-7543

QP501.C5
Adonis

STIC-ILL

From: Portner, Ginny
Sent: Thursday, January 02, 2003 11:35 AM
To: STIC-ILL
Subject: 09/727,892 (adonis reference)

Importance: High

TITLE: Bioluminescence-based assays for detection and characterization of bacteria and chemicals in clinical laboratories
AUTHOR(S): Billard P; DuBow MS (REPRINT)
CORPORATE SOURCE: UNIV METZ,CTR ENVIRONM SCI, 1 RUE RECOLLETS, BP 94025/F-57040 METZ//FRANCE/ (REPRINT); UNIV METZ,CTR ENVIRONM SCI/F-57040 METZ//FRANCE/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CLINICAL BIOCHEMISTRY, 1998, V31, N1 (FEB), P1-14
GENUINE ARTICLE#: ZE635
PUBLISHER: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB
ISSN: 0009-9120
CURRENT CONTENTS JOURNAL ANNOUNCEMENT: CC LIFE, V41, N17
LANGUAGE: English DOCUMENT TYPE: REVIEW
GEOGRAPHIC LOCATION: FRANCE
SUBFILE: SciSearch; CC LIFE--Current Contents/Life Sciences
JOURNAL SUBJECT CATEGORY: MEDICAL RESEARCH, DIAGNOSIS & TREATMENT

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(703) 308-7543

STIC-ILL 9184165 V ND

From: Portner, Ginny
Sent: Thursday, January 02, 2003 2:06 PM
To: STIC-ILL
Subject: 09/727,892

Importance: High

426/80

02583615 BIOSIS NO.: 000017031673
EARLY FUNCTION OF A VIRULENT STAPHYLOCOCCAL PHAGE
AUTHOR: LATHAM J M; HARRIS E F
JOURNAL: ABSTR ANNU MEET AM SOC MICROBIOL (79). 1979 281 1979
FULL JOURNAL NAME: Abstracts of the Annual Meeting of the American Society for Microbiology
CODEN: ASMAC
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
DESCRIPTORS: ABSTRACT PHAGE 44A- HJD STAPHYLOCOCCUS - AUREUS COVALENTLY CLOSED CIRCULAR DNA

0094-8519

01074744 BIOSIS NO.: 000009054954
EFFECTS OF INCREASED SERINE CONCENTRATION IN THE CELL WALL OF STAPHYLOCOCCUS - AUREUS 44A HJD
AUTHOR: DONEGAN E A; RIGGS H G JR
JOURNAL: ABSTR ANNU MEET AM SOC MICROBIOL 73. 1973 182 1973
FULL JOURNAL NAME: Abstracts of the Annual Meeting of the American Society for Microbiology
CODEN: ASMAC
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
DESCRIPTORS: ABSTRACT

00633656 BIOSIS NO.: 000007083621
BACTERIO PHAGE REPRODUCTION IN LYSOSTAPHIN TREATED STAPHYLOCOCCUS - AUREUS 44-A- HJD
AUTHOR: ONDERDONK A B; RIGGS H G JR
JOURNAL: BACTERIOL PROC 71. 1971 185 1971
FULL JOURNAL NAME: Bacteriological Proceedings
CODEN: BACPA
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
DESCRIPTORS: ABSTRACT PROTOPLAST ANTI INFECT-DRUG ELECTRON MICROSCOPE PHOSPHORUS-32 DNA
CONCEPT CODES:

Ginny Portner
CM1, Art Unit 1645
Room 7e13
Mail box 7e12
(703) 308-7543

STIC-ILL 9184149 V NO

From: Portner, Ginny
Sent: Thursday, January 02, 2003 2:06 PM
To: STIC-ILL
Subject: 09/727,892

Importance: High

426187

02583615 BIOSIS NO.: 000017031673
EARLY FUNCTION OF A VIRULENT STAPHYLOCOCCAL PHAGE
AUTHOR: LATHAM J M; HARRIS E F
JOURNAL: ABSTR ANNU MEET AM SOC MICROBIOL (79). 1979 281 1979
FULL JOURNAL NAME: Abstracts of the Annual Meeting of the American Society for Microbiology
CODEN: ASMAC
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
DESCRIPTORS: ABSTRACT PHAGE 44A- HJD STAPHYLOCOCCUS - AUREUS COVALENTLY CLOSED CIRCULAR DNA

01074744 BIOSIS NO.: 000009054954
EFFECTS OF INCREASED SERINE CONCENTRATION IN THE CELL WALL OF STAPHYLOCOCCUS - AUREUS 44A HJD
AUTHOR: DONEGAN E A; RIGGS H G JR
JOURNAL: ABSTR ANNU MEET AM SOC MICROBIOL 73. 1973 182 1973
FULL JOURNAL NAME: Abstracts of the Annual Meeting of the American Society for Microbiology
CODEN: ASMAC
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
DESCRIPTORS: ABSTRACT

0094-8519

00633656 BIOSIS NO.: 000007083621
BACTERIO PHAGE REPRODUCTION IN LYSOSTAPHIN TREATED STAPHYLOCOCCUS AUREUS 44-A- HJD
AUTHOR: ONDERDONK A B; RIGGS H G JR
JOURNAL: BACTERIOL PROC 71. 1971 185 1971
FULL JOURNAL NAME: Bacteriological Proceedings
CODEN: BACPA
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
DESCRIPTORS: ABSTRACT PROTOPLAST ANTI INFECT-DRUG ELECTRON MICROSCOPE PHOSPHORUS-32 DNA
CONCEPT CODES:

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STIC-ILL

NO 4262777

From: Portner, Ginny
Sent: Thursday, January 02, 2003 3:03 PM
To: STIC-ILL
Subject: FW: 09/727,892

Importance: High

A Book: Methods in Molecular Biology; Drug-DNA interaction protocols
Author: Dooley, Thomas P et al
Editor: Fox, KR: Ed
Journal: Methods in Molecular Microbiology, 90, page 117-12, 1997
ISSN:0097-0816

Ginny Portner
CM1, Art Unit 1645
Room 7e13
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09/727892

Inhibitors of DNA polymerase III as novel antimicrobial agents against gram-positive eubacteria.

AUTHOR: Tarantino Paul M Jr; Zhi Chengxin; Wright George E; Brown Neal C(a)
AUTHOR ADDRESS: (a)Dept. of Pharmacology and Molecular Toxicology, University of Massachusetts Medical School, Worc**USA

JOURNAL: Antimicrobial Agents and Chemotherapy 43 (8):p1982-1987 Aug., 1999
ISSN: 0066-4804
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: 6-Anilinouracils are selective inhibitors of DNA polymerase III, the enzyme required for the replication of chromosomal DNA in gram-positive bacteria (N. C. Brown, L. W. Dudycz, and G. E. Wright, Drugs Exp. Clin. Res. 12:555-564, 1986). A new class of 6-anilinouracils based on N-3 alkyl substitution of the uracil ring was synthesized and analyzed for activity as inhibitors of the gram-positive bacterial DNA polymerase III and the growth of gram-positive bacterial pathogens. Favorable in vitro properties of N-3-alkyl derivatives prompted the synthesis of derivatives in which the R group at N-3 was replaced with more-hydrophilic methoxyalkyl and hydroxyalkyl groups. These hydroxyalkyl and methoxyalkyl derivatives displayed Ki values in the range from 0.4 to 2.8 muM against relevant gram-positive bacterial DNA polymerase IIIs and antimicrobial activity with MICs in the range from 0.5 to 15 mug/ml against a broad spectrum of gram-positive bacteria, including methicillin-resistant staphylococci and vancomycin-resistant enterococci. Two of these hydrophilic derivatives displayed protective activity in a simple mouse model of lethal staphylococcal infection.

REGISTRY NUMBERS: 7269-15-0: 6-ANILINOURACILS; 37217-33-7: DNA POLYMERASE

1/9/12 (Item 6 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

09346349 References: 72
TITLE: Bioluminescence-based assays for detection and characterization of bacteria and chemicals in clinical laboratories
AUTHOR(S): Billard P; **DuBow MS (REPRINT)**
CORPORATE SOURCE: UNIV METZ,CTR ENVIRONM SCI, 1 RUE RECOLLETS, BP 94025/F-57040 METZ//FRANCE/ (REPRINT); UNIV METZ,CTR ENVIRONM SCI/F-57040 METZ//FRANCE/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CLINICAL BIOCHEMISTRY, 1998, V31, N1 (FEB), P1-14
GENUINE ARTICLE#: ZE635
PUBLISHER: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB
ISSN: 0009-9120
CURRENT CONTENTS JOURNAL ANNOUNCEMENT: CC LIFE, V41, N17
LANGUAGE: English DOCUMENT TYPE: REVIEW
GEOGRAPHIC LOCATION: FRANCE
SUBFILE: SciSearch; CC LIFE--Current Contents/Life Sciences
JOURNAL SUBJECT CATEGORY: MEDICAL RESEARCH, DIAGNOSIS & TREATMENT

ABSTRACT: Objectives: To survey recent advances in the application of bioluminescence to public health problems. The usefulness of bacterial (lux) and eucaryotic (luc) luciferase genes is presented, along with several examples that demonstrate their value as ''reporters'' of many endpoints of clinical concern.

Conclusions: The development of new technologies for monitoring biological and chemical contaminants is in continuous progress. Recent excitment in this area has come from the use of genes encoding enzymes for bioluminescence as reporter systems. Applications of the recombinant luciferase reporter **phage** concept now provide a sensitive approach for bacterial detection, their viability, and sensitivity to antimicrobial agents. Moreover, a number of fusions of the lux and luc genes to stress inducible genes in different bacteria can allow a real-time measurement of gene expression and determination of cellular viability, and also constitute a new tool to detect toxic chemicals and their bioavailibility.
DESCRIPTORS--Author Keywords: bioluminescence ; luciferase ; biosensor ; reporter ; **phages** ; bacterial pathogens ; chemicals ; detection
IDENTIFIERS--KeyWord Plus: GREEN-FLUORESCENT PROTEIN; CHROMOSOMAL ARS OPERON; ESCHERICHIA-COLI; FIREFLY LUCIFERASE; GENE FUSIONS; **STAPHYLOCOCCUS** - **AUREUS** ; SALMONELLA-TYPHIMURIUM; TRANSIENT TRANSFECTION; SENSITIVE DETECTION; DIFFERENT COLORS

In vitro incorporation of serine into the staphylococcal cell wall

Donegan E.A.; Riggs Jr H.G.
Dept. Microbiol., Univ. Missouri Sch. Med., Columbia, Mo. 65201 United States

Infection and Immunity (INFECT. IMMUN.) 1974, 10/1 (264-269)
CODEN: INFIB
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH

A variant of S. **aureus** 44A **HJD** was isolated by serial growth in Trypticase soy broth to which 2 M serine had been added (wt/vol). Amino acid analysis of hydrolysates of purified mucopeptides from the variant showed that they contained 1.266 serine and 2.156 glycine residues per glutamic acid residue, compared with 0.174 serine and 3.144 glycine residues per glutamic acid residue in the mucopeptide of the parent strain. In addition to this alteration in the chemical composition of the mucopeptide, the variant lost many of the biochemical and cultural characteristics of the parent organism. The variant was not sensitive to the lytic action of lysostaphin and was non phage typable. Moreover, in vitro tests indicated that the organism was coagulase negative, did not produce gelatinase or deoxyribonuclease, and did not hemolyze sheep erythrocytes. Apparently due to the change in the serine content in the cell wall of the parent S. aureus strain, the organism had become 'epidermidis like' in its properties.

11232799 BIOSIS NO.: 199800014131

DNA polymerase inhibition assay (PIA) for the detection of drug-DNA interactions.

BOOK TITLE: Methods in Molecular Biology; Drug-DNA interaction protocols

AUTHOR: Dooley Thomas P(a); Weiland Katherine L
BOOK AUTHOR/EDITOR: Fox K R: Ed
AUTHOR ADDRESS: (a)Molecular Pharmacol., Southern Res. Inst., Birmingham, AL**USA

JOURNAL: Methods in Molecular Biology 90p117-125 1997
BOOK PUBLISHER: Humana Press Inc., Suite 808, 999 Riverview Drive, Totowa, New Jersey 07512, USA
ISSN: 0097-0816 ISBN: 0-89603-447-X
DOCUMENT TYPE: Book
RECORD TYPE: Citation
LANGUAGE: English
REGISTRY NUMBERS: 9012-90-2: DNA POLYMERASE

DESCRIPTORS:
- MAJOR CONCEPTS: Methods and Techniques; Molecular Genetics (Biochemistry and Molecular Biophysics); Pharmacology
- CHEMICALS & BIOCHEMICALS: drug; DNA--drug interaction
- METHODS & EQUIPMENT: **DNA polymerase inhibition assay** --analytical method
- MISCELLANEOUS TERMS: Book Chapter

CONCEPT CODES:
- 10050 Biochemical Methods-General
- 03502 Genetics and Cytogenetics-General
- 10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines
- 10060 Biochemical Studies-General
- 10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
- 10804 Enzymes-Methods

09346349 References: 72
TITLE: Bioluminescence-based assays for detection and characterization of bacteria and chemicals in clinical laboratories
AUTHOR(S): Billard P; **DuBow MS (REPRINT)**
CORPORATE SOURCE: UNIV METZ,CTR ENVIRONM SCI, 1 RUE RECOLLETS, BP 94025/F-57040 METZ//FRANCE/ (REPRINT); UNIV METZ,CTR ENVIRONM SCI/F-57040 METZ//FRANCE/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CLINICAL BIOCHEMISTRY, 1998, V31, N1 (FEB), P1-14
GENUINE ARTICLE#: ZE635
PUBLISHER: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB
ISSN: 0009-9120
CURRENT CONTENTS JOURNAL ANNOUNCEMENT: CC LIFE, V41, N17
LANGUAGE: English DOCUMENT TYPE: REVIEW
GEOGRAPHIC LOCATION: FRANCE
SUBFILE: SciSearch; CC LIFE--Current Contents/Life Sciences
JOURNAL SUBJECT CATEGORY: MEDICAL RESEARCH, DIAGNOSIS & TREATMENT

ABSTRACT: Objectives: To survey recent advances in the application of bioluminescence to public health problems. The usefulness of bacterial (lux) and eucaryotic (luc) luciferase genes is presented, along with several examples that demonstrate their value as ''reporters'' of many endpoints of clinical concern.

Conclusions: The development of new technologies for monitoring biological and chemical contaminants is in continuous progress. Recent excitment in this area has come from the use of genes encoding enzymes for bioluminescence as reporter systems. Applications of the recombinant luciferase reporter **phage** concept now provide a sensitive approach for bacterial detection, their viability, and sensitivity to antimicrobial agents. Moreover, a number of fusions of the lux and luc genes to stress inducible genes in different bacteria can allow a real-time measurement of gene expression and determination of cellular viability, and also constitute a new tool to detect toxic chemicals and their bioavailibility.
DESCRIPTORS--Author Keywords: bioluminescence ; luciferase ; biosensor ; reporter ; **phages** ; bacterial pathogens ; chemicals ; detection
IDENTIFIERS--KeyWord Plus: GREEN-FLUORESCENT PROTEIN; CHROMOSOMAL ARS OPERON; ESCHERICHIA-COLI; FIREFLY LUCIFERASE; GENE FUSIONS; **STAPHYLOCOCCUS - AUREUS** ; SALMONELLA-TYPHIMURIUM; TRANSIENT TRANSFECTION; SENSITIVE DETECTION; DIFFERENT COLORS

3/9/15 (Item 15 from file: 156)
DIALOG(R)File 156:ToxFile

01259182 98297420 PMID: 9635497

An ergosterol peroxide, a natural product that selectively enhances the inhibitory effect of linoleic acid on DNA polymerase beta.

Mizushina Y; Watanabe I; Togashi H; Hanashima L; Takemura M; Ohta K; Sugawara F; Koshino H; Esumi Y; Uzawa J; Matsukage A; Yoshida S; Sakaguchi K

Department of Applied Biological Science, Science University of Tokyo, Chiba, Japan.

Biological & pharmaceutical bulletin (JAPAN) May 1998, 21 (5) p444-8 , ISSN 0918-6158 Journal Code: 9311984

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: Toxbib ; INDEX MEDICUS

As described previously (Mizushina Y., Tanaka N., Yagi H., Kurosawa T., Onoue M., Seto H., Horie T., Aoyagi N., Yamaoka M., Matsukage A., Yoshida S., and Sakaguchi K., Biochim. Biophys. Acta, 1308, 256-262, 1996), linoleic acid (LA) inhibits the activities of mammalian DNA polymerases. We found a natural product from a basidiomycete, Ganoderma lucidum, that enhances this effect of LA in a special manner. The structure was identified to be an ergosterol peroxide, 5,8-epidioxy-5alpha,8alpha-ergosta -6,22E-dien -3beta-ol by spectroscopic analyses. The ergosterol peroxide (EPO) itself scarcely inhibited the activities of calf thymus DNA polymerase alpha (pol. alpha) or rat DNA polymerase beta (pol. beta). However, when EPO at 0.25 mM was present, 10 microM or less of LA almost completely inhibited the pol. beta activity, while almost complete inhibition by LA itself was achieved at 80 microM or higher. Interestingly, under the same conditions, EPO did not affect the LA-effect on pol. alpha. The action mode of the EPO was discussed.

Tags: Animal; Support, Non-U.S. Gov't

Descriptors: **DNA Polymerase beta** --antagonists and inhibitors--AI; *Enzyme Inhibitors--pharmacology--PD; *Ergosterol--analogs and derivatives --AA; *Linoleic Acid--pharmacology--PD; Basidiomycota--enzymology--EN; Cattle; Chromatography, High Pressure Liquid; DNA Polymerase I--antagonists and inhibitors--AI; Drug Synergism; Ergosterol--pharmacology--PD; Fatty Acids--chemistry--CH; Fatty Acids--metabolism--ME; Rats

CAS Registry No.: 0 (Enzyme Inhibitors); 0 (Fatty Acids); 2061-64-5 (ergosterol-5,8-peroxide); 2197-37-7 (Linoleic Acid); 57-87-4 (Ergosterol)

Enzyme No.: EC 2.7.7.- (DNA Polymerase I); EC 2.7.7.- (DNA Polymerase beta)

Record Date Created: 19980824

3/9/16 (Item 16 from file: 156)
DIALOG(R)File 156:ToxFile

01252163 98250009 PMID: 9590127

Sulfated glycoglycerolipid from archaebacterium inhibits eukaryotic DNA polymerase alpha, beta and retroviral reverse transcriptase and affects methyl methanesulfonate cytotoxicity.

Ogawa A; Murate T; Izuta S; Takemura M; Furuta K; Kobayashi J; Kamikawa T ; Nimura Y; Yoshida S

First Department of Surgery, Nagoya University School of Medicine, Japan.

International journal of cancer. Journal international du cancer (UNITED STATES) May 18 1998, 76 (4) p512-8, ISSN 0020-7136 Journal Code: 0042124

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: Toxbib ; INDEX MEDICUS; AIDS/HIV

A sulfated glycoglycerolipid, 1-O-(6'-sulfo-alpha-D-glucopyranosyl)-2,3-d

i-O-phytanyl- sn-glycerol (KN-208), a derivative of the polar lipid isolated from an archaebacterium, strongly inhibited DNA polymerase (pol) alpha and pol beta in vitro among 5 eukaryotic DNA polymerases (alpha, beta, gamma, delta, and epsilon). It also inhibited Escherichia coli DNA polymerase I Klenow fragment (E. coli pol I) and human immunodeficiency virus reverse transcriptase (HIV RT). The mode of inhibition of these polymerases was competitive with the DNA template primer and was non-competitive with the substrate dTTP. KN-208 inhibited pol beta most strongly, with a Ki value of 0.05 microM, 10-fold lower than that for pol alpha (0.5 microM) and 60- or 140-fold lower than that for HIV RT (3 microM) or for E. coli pol I (7 microM), respectively. The loss of sulfate on the 6'-position of glucopyranoside of this compound completely abrogated inhibition. However, the hydrophilic part of KN-208, glucose 6-sulfate alone, showed no inhibition. Other sulfated compounds containing different hydrophobic structures, such as dodecyl sulfate and cholesterol sulfate, exhibited a much weaker inhibition. Our results suggest that the whole molecular structure of KN-208 is required for inhibition. KN-208 was shown to be modestly cytotoxic for the human leukemic cell line K562. Interestingly, a subcytotoxic dose of KN-208 increased the sensitivity of the human leukemic cells to an alkylating agent, methyl methanesulfonate, while it did not potentiate the effects of ultraviolet light or of cisplatin.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: *Archaea--enzymology--EN; *DNA-Directed DNA Polymerase --antagonists and inhibitors--AI; *Glycolipids--pharmacology--PD; *HIV --enzymology--EN; *Methyl Methanesulfonate--pharmacology--PD; *Reverse Transcriptase Inhibitors; Cell Division; DNA Polymerase I--antagonists and inhibitors--AI; **DNA Polymerase beta** --antagonists and inhibitors--AI; Glycolipids--chemistry--CH; Leukemia, Experimental; Tumor Cells, Cultured

CAS Registry No.: 0 (1-O-(6'-sulfo-alpha-D-glucopyranosyl)-2,3-di-O-phyt anyl-sn-glycerol); 0 (Glycolipids); 0 (Reverse Transcriptase Inhibitors) ; 66-27-3 (Methyl Methanesulfonate)

Enzyme No.: EC 2.7.7.- (DNA Polymerase I); EC 2.7.7.- (DNA Polymerase beta); EC 2.7.7.7 (DNA-Directed DNA Polymerase)

Record Date Created: 19980604

?t s13/9/23 26 46

13/9/23 (Item 23 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

12109422 BIOSIS NO.: 199900404271

Inhibitors of DNA polymerase III as novel antimicrobial agents against gram-positive eubacteria.

AUTHOR: Tarantino Paul M Jr; Zhi Chengxin; Wright George E; Brown Neal C(a)

AUTHOR ADDRESS: (a)Dept. of Pharmacology and Molecular Toxicology, University of Massachusetts Medical School, Worc**USA

JOURNAL: Antimicrobial Agents and Chemotherapy 43 (8):p1982-1987 Aug., 1999

ISSN: 0066-4804

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

SUMMARY LANGUAGE: English

ABSTRACT: 6-Anilinouracils are selective inhibitors of DNA polymerase III, the enzyme required for the replication of chromosomal DNA in gram-positive bacteria (N. C. Brown, L. W. Dudycz, and G. E. Wright, Drugs Exp. Clin. Res. 12:555-564, 1986). A new class of 6-anilinouracils based on N-3 alkyl substitution of the uracil ring was synthesized and analyzed for activity as inhibitors of the gram-positive bacterial DNA polymerase III and the growth of gram-positive bacterial pathogens. Favorable in vitro properties of N-3-alkyl derivatives prompted the synthesis of derivatives in which the R group at N-3 was replaced with more-hydrophilic methoxyalkyl and hydroxyalkyl groups. These hydroxyalkyl and methoxyalkyl derivatives displayed Ki values in the range from 0.4 to 2.8 muM against relevant gram-positive bacterial DNA polymerase IIIs and antimicrobial activity with MICs in the range from 0.5 to 15 mug/ml against a broad spectrum of gram-positive bacteria, including

methicillin-resistant staphylococci and vancomycin-resistant enterococci. Two of these hydrophilic derivatives displayed protective activity in a simple mouse model of lethal staphylococcal infection.

REGISTRY NUMBERS: 7269-15-0: 6-ANILINOURACILS; 37217-33-7: DNA POLYMERASE III
DESCRIPTORS:
MAJOR CONCEPTS: Enzymology (Biochemistry and Molecular Biophysics); Infection; Pharmacology
BIOSYSTEMATIC NAMES: Gram-Positive Cocci--Eubacteria, Bacteria, Microorganisms; Micrococcaceae--Gram-Positive Cocci, Eubacteria, Bacteria, Microorganisms; Muridae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: enterococci (Gram-Positive Cocci)--pathogen, vancomycin-resistant; mouse (Muridae)--model; staphylococci (Micrococcaceae)--methicillin-resistant, pathogen
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Animals; Bacteria; Chordates; Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates
DISEASES: staphylococcal infection--bacterial disease
CHEMICALS & BIOCHEMICALS: DNA polymerase III; 6-anilinouracils-- **DNA polymerase III inhibitor** , antimicrobial activity
CONCEPT CODES:
38504 Chemotherapy-Antibacterial Agents
10060 Biochemical Studies-General
10802 Enzymes-General and Comparative Studies; Coenzymes
12512 Pathology, General and Miscellaneous-Therapy (1971-)
13002 Metabolism-General Metabolism; Metabolic Pathways
36002 Medical and Clinical Microbiology-Bacteriology
BIOSYSTEMATIC CODES:
07700 Gram-Positive Cocci (1992-)
07702 Micrococcaceae (1992-)
86375 Muridae

13/9/26 (Item 26 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11898957 BIOSIS NO.: 199900145066
Nucleic acid ligand inhibitors to DNA polymerases.
AUTHOR: Gold L; Jayasena S D
AUTHOR ADDRESS: Boulder, Colo.**USA
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents 1219 (4):p3394 Feb. 23, 1999
PATENT NUMBER: US 5874557 PATENT DATE GRANTED: Feb. 23, 1999 19990223
PATENT ASSIGNEE: NEXSTAR PHARMACEUTICALS, INC. PATENT COUNTRY: USA
ISSN: 0098-1133
RECORD TYPE: Citation
LANGUAGE: English
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and Molecular Biophysics); Pharmacology
MISCELLANEOUS TERMS: Patent; BIOTECHNOLOGY; **DNA POLYMERASE INHIBITOR** ; ENZYME INHIBITOR; NUCLEIC ACID LIGAND; PHARMACEUTICALS

13/9/46 (Item 46 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10453687 BIOSIS NO.: 199699074832
An application of MTT-colorimetric assay for the screening of anti-adenovirus agents.
AUTHOR: Kodama E(a); Shigeta S; Suzuki T; De Clercq E
AUTHOR ADDRESS: (a)Dep. Pediatr., Univ. Alabama at Birmingham, AL**USA
JOURNAL: Antiviral Research 30 (1):pA54 1996
CONFERENCE/MEETING: Ninth International Conference on Antiviral Research Urabandai, Japan May 19-24, 1996
ISSN: 0166-3542

RECORD TYPE: Citation
LANGUAGE: English
REGISTRY NUMBERS: 9012-90-2: DNA POLYMERASE
DESCRIPTORS:
  MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Cell Biology; Infection; Methods and Techniques; Pathology; Pharmacology
  BIOSYSTEMATIC NAMES: Adenoviridae--Viruses; Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
  ORGANISMS: Adenoviridae (Adenoviridae); Hominidae (Hominidae)
  BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): animals; chordates; humans; mammals; microorganisms; primates; vertebrates; viruses
  CHEMICALS & BIOCHEMICALS: DNA POLYMERASE
  MISCELLANEOUS TERMS: ANTIVIRAL-DRUG; **DNA POLYMERASE INHIBITOR** ; HUMAN MKN-28 CELLS; MEETING ABSTRACT; MEETING POSTER; SCREENING METHOD
CONCEPT CODES:
  10010 Comparative Biochemistry, General
  10504 Biophysics-General Biophysical Techniques
  10506 Biophysics-Molecular Properties and Macromolecules
  12512 Pathology, General and Miscellaneous-Therapy (1971-)
  22002 Pharmacology-General
  22003 Pharmacology-Drug Metabolism; Metabolic Stimulators
  32600 In Vitro Studies, Cellular and Subcellular
  36006 Medical and Clinical Microbiology-Virology
  38506 Chemotherapy-Antiviral Agents
  00520 General Biology-Symposia, Transactions and Proceedings of Conferences, Congresses, Review Annuals
  10060 Biochemical Studies-General
  10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
  10064 Biochemical Studies-Proteins, Peptides and Amino Acids
  10808 Enzymes-Physiological Studies
  13012 Metabolism-Proteins, Peptides and Amino Acids
  13014 Metabolism-Nucleic Acids, Purines and Pyrimidines
  14001 Digestive System-General; Methods
  24005 Neoplasms and Neoplastic Agents-Neoplastic Cell Lines
  32500 Tissue Culture, Apparatus, Methods and Media
  33506 Virology-Animal Host Viruses
BIOSYSTEMATIC CODES:
  02601 Adenoviridae (1993-)
  86215 Hominidae
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### Status: Signed Off. (1 minutes)
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10389336 99353975 PMID: 10423540

The cyanogenic glucoside, prunasin (D-mandelonitrile-beta-D-glucoside), is a novel inhibitor of DNA polymerase beta.

Mizushina Y; Takahashi N; Ogawa A; Tsurugaya K; Koshino H; Takemura M; Yoshida S; Matsukage A; Sugawara F; Sakaguchi K

Department of Applied Biological Science, Science University of Tokyo, Noda, Chiba, 278-8510, Japan.

Journal of biochemistry (JAPAN) Aug 1999, 126 (2) p430-6, ISSN 0021-924X Journal Code: 0376600

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A DNA polymerase beta (pol. beta) inhibitor has been isolated independently from two organisms; a red perilla, Perilla frutescens, and a mugwort, Artemisia vulgaris. These molecules were determined by spectroscopic analyses to be the cyanogenic glucoside, D-mandelonitrile-beta-D-glucoside, prunasin. The compound inhibited the activity of rat pol. beta at 150 microM, but did not influence the activities of calf DNA polymerase alpha and plant DNA polymerases, human immunodefficiency virus type 1 reverse transcriptase, calf terminal deoxynucleotidyl transferase, or any prokaryotic DNA polymerases, or DNA and RNA metabolic enzymes examined. The compound dose-dependently inhibited pol. beta activity, the IC(50) value being 98 microM with poly dA/oligo dT(12-18) and dTTP as the DNA template and substrate, respectively. Inhibition of pol. beta by the compound was competitive with the substrate, dTTP. The inhibition was enhanced in the presence of fatty acid, and the IC(50) value decreased to approximately 40 microM. In the presence of C(10)-decanoic acid, the K(i) value for substrate dTTP decreased by 28-fold, suggesting that the fatty acid allowed easier access of the compound to the substrate-binding site.

Tags: Animal; Comparative Study; Human; Support, Non-U.S. Gov't

Descriptors: **DNA Polymerase beta** --antagonists and inhibitors--AI; *Enzyme Inhibitors--chemistry--CH; *Enzyme Inhibitors--pharmacokinetics --PK; *Nitriles--chemistry--CH; *Nitriles--pharmacokinetics--PK; Amygdalin --chemistry--CH; Amygdalin--pharmacokinetics--PK; Artemisia--chemistry--CH ; Artemisia--enzymology--EN; Cattle; Decanoic Acids--pharmacology--PD; Dose-Response Relationship, Drug; Enzyme Inhibitors --isolation and purification--IP; Inhibitory Concentration 50; Kinetics; Lamiaceae --chemistry--CH; Nitriles--isolation and purification--IP; Plants, Medicinal; Rats; Thymine Nucleotides--chemistry--CH

CAS Registry No.: 0 (Decanoic Acids); 0 (Enzyme Inhibitors); 0 (Nitriles); 0 (Thymine Nucleotides); 138-53-4 (prunasin); 29883-15-6 (Amygdalin); 334-48-5 (decanoic acid); 611-60-9 (2',3'-dideoxythymidine triphosphate)

Enzyme No.: EC 2.7.7.- (DNA Polymerase beta)

Record Date Created: 19991213

10387061 99355950 PMID: 10425125

Harbinatic acid, a novel and potent DNA polymerase beta inhibitor from Hardwickia binata.

Deng J Z; Starck S R; Hecht S M; Ijames C F; Hemling M E

Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22901, USA.

Journal of natural products (UNITED STATES) Jul 1999, 62 (7) p1000-2 , ISSN 0163-3864 Journal Code: 7906882

Contract/Grant No.: CA50771; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Bioassay-guided fractionation of an active methyl ethyl ketone extract of Hardwickia binata, using an assay sensitive to DNA polymerase beta inhibition, resulted in the isolation of a potent inhibitor. This proved to be a novel diterpenoid, which has been named harbinatic acid (1). The structure of 1 was established as 3alpha-O-trans-p-coumaroyl-7-labden-15-oi c acid from spectroscopic analysis and by comparison with the published data for a structurally related compound. Compound 1 strongly inhibited calf thymus DNA polymerase beta, with an IC(50) value of 2.9 &mgr;M.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: **DNA Polymerase beta** --antagonists and inhibitors--AI; *Diterpenes--isolation and purification--IP; *Enzyme Inhibitors--isolation and purification--IP; *Fabaceae--chemistry--CH; *Plants, Medicinal; Diterpenes--pharmacology--PD; Enzyme Inhibitors--pharmacology--PD; India; Magnetic Resonance Spectroscopy; Plant Extracts--chemistry--CH; Plant Extracts--pharmacology--PD

CAS Registry No.: 0 (Diterpenes); 0 (Enzyme Inhibitors); 0 (Plant Extracts); 0 (harbinatic acid)

Enzyme No.: EC 2.7.7.- (DNA Polymerase beta)

Record Date Created: 19991015

10233734 99197137 PMID: 10096862

bis-5-Alkylresorcinols from Panopsis rubescens that inhibit DNA polymerase beta.

Deng J Z; Starck S R; Hecht S M

Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901, USA.

Journal of natural products (UNITED STATES) Mar 1999, 62 (3) p477-80 , ISSN 0163-3864 Journal Code: 7906882

Contract/Grant No.: CA50771; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Bioassay-guided fractionation of Panopsis rubescens, using an assay to detect DNA polymerase beta inhibition, led to the isolation of two new bis-5-alkylresorcinols (1 and 2), in addition to one known bis-5-alkylresorcinol (3). The structures of 1-3 were established as 1,3-dihydroxy-5-[14'-(3' ',5' '-dihydroxyphenyl)-cis-4'-tetradecenyl]benzene (1), 1, 3-dihydroxy-5-[14'-(3' ',5' '-dihydroxyphenyl)-cis-7'-tetradecenyl]benzene (2), and 1, 3-dihydroxy-5-[14'-(3' ',5' '-dihydroxyphenyl)tetradecenyl]benzene (3), respectively, by spectroscopic and chemical analyses. Compounds 1-3 exhibited potent inhibition of calf thymus DNA polymerase beta, with IC50 values of 7.5, 6.5, and 5.8 microM, respectively.

Tags: Animal; Support, U.S. Gov't, P.H.S.

Descriptors: **DNA Polymerase beta** --antagonists and inhibitors--AI; *Enzyme Inhibitors--chemistry--CH; *Plants, Medicinal--chemistry--CH; *Resorcinols--chemistry--CH; Acetylation; Cattle; Enzyme Inhibitors --isolation and purification--IP; Magnetic Resonance Spectroscopy; Plant Extracts--chemistry--CH; Plant Stems--chemistry--CH; Resorcinols--isolation and purification--IP; Spectrometry, Mass, Fast Atom Bombardment

CAS Registry No.: 0 (Enzyme Inhibitors); 0 (Plant Extracts); 0 (Resorcinols)

Enzyme No.: EC 2.7.7.- (DNA Polymerase beta)

Record Date Created: 19990607

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S5          2   'DNA POLYMERASE III BETA SUBUNIT DNAN GENE' OR 'DNA POLYME-
             RASE III BETA-SUBUNIT'
S6          2   'DNA POLYMERASE III INHIBITOR'
S7         75   E27-E31
S8          1   'DNA POLYMERASE INHIBITION ASSAY'
S9          1   'DNA POLYMERASE POL' OR S27
S10         1   'DNA POLYMERASE STOP ASSAY'
S11         3   'DNA POLYMERASE SUBUNIT'
S12       219   E24-E41
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            OR S11 OR S12)
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S15        62   RD (unique items)
S16  15869122   POL3 OR POLLLL OR (POL (2N) 111 OR III OR LLL OR 3)
S17  15870721   S13 OR S16 OR DNAN
S18   3107853   S17 AND (ANTIBACT? OR ANTIBIO? OR ANTIMICROB? OR ANTAGON? -
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S20      3109   S19 AND (BACTERIOPHAG? OR PHAGE?)
S21     64210   S17 AND (BACTERIOPHAG? OR PHAGE?)
S22      1331   S19 AND S20 AND S18
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?e holin

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E2        1  HOLIMUM
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E8        1  HOLIN HOL GENE
E9        1  HOLIN HOL PROTEIN
E10       1  HOLIN HOMOLOGY
E11       2  HOLIN INHIBITOR
E12       1  HOLIN INTEGRAL MEMBRANE PROTEINS

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>>>Year ranges not supported in one or more files
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relevant records.
Searching ALL records
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...Processed 20   out of 27  files
...Processing Complete
     S32     50  TARGET - S31
Ending TARGET search. Enter TARGET to do another search in the present
file(s), or BEGIN new file(s).  Enter LOGOFF to disconnect from Dialog
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Printed

15/9/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09674800 98101633 PMID: 9440683
A mechanism for all polymerases.
Steitz T A
Nature (ENGLAND) Jan 15 1998, 391 (6664) p231-2, ISSN 0028-0836 Journal Code: 0410462
Comment on Nature. 1998 Jan 15;391(6664) 251-8; Comment on PMID 9440688; Comment on Nature. 1998 Jan 15;391(6664):304-7; Comment on PMID 9440698
Document type: Comment; News
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Descriptors: *DNA-Directed DNA Polymerase--metabolism--ME; Bacillus stearothermophilus--enzymology--EN; Catalysis; Crystallography, X-Ray; DNA --metabolism--ME; **DNA Polymerase beta** --chemistry--CH; **DNA Polymerase beta** --metabolism--ME; DNA-Directed DNA Polymerase--chemistry--CH; Escherichia coli--metabolism--ME; Magnesium--chemistry--CH; Magnesium --metabolism--ME; Models, Molecular; Protein Conformation; Thioredoxin --metabolism--ME
CAS Registry No.: 52500-60-4 (Thioredoxin); 7439-95-4 (Magnesium); 9007-49-2 (DNA)
Enzyme No.: EC 2.7.7.- (DNA Polymerase beta); EC 2.7.7.- (**bacteriophage** T7 induced DNA polymerase); EC 2.7.7.7 (DNA-Directed DNA Polymerase)
Record Date Created: 19980130

00510535 (THIS IS THE FULLTEXT)

Genetic Selection of Peptide Inhibitors of Biological Pathways

Norman, Thea C.<CRF RID="C1"> ; Smith, Dana L.; Sorger, Peter K.; Drees, Becky L.; O'Rourke, Sean M.; Hughes, Timothy R.; Roberts, Christopher J.; Friend, Stephen H.; Fields, Stan; Murray, Andrew W.

Department of Physiology, University of California, San Francisco, CA 94143-0444, USA. Department of Genetics and Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, USA. Department of Biochemistry, University of California, San Francisco, CA 94143-0448, USA. Rosetta Inpharmatics, Kirkland, WA 98034, USA.

Science Vol. 285 5427 pp. 591
Publication Date: 7-23-1999 (990723) Publication Year: 1999
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: REPORTS
Word Count: 3728

Abstract: Genetic selections were used to find peptides that inhibit biological pathways in budding yeast. The peptides were presented inside cells as peptamers, surface loops on a highly expressed and biologically inert carrier protein, a catalytically inactive derivative of staphylococcal nuclease. Peptamers that inhibited the pheromone signaling pathway, transcriptional silencing, and the spindle checkpoint were isolated. Putative targets for the inhibitors were identified by a combination of two-hybrid analysis and genetic dissection of the target pathways. This analysis identified Ydr517w as a component of the spindle checkpoint and reinforced earlier indications that Ste50 has both positive and negative roles in pheromone signaling. Analysis of transcript arrays showed that the peptamers were highly specific in their effects, which suggests that they may be useful reagents in organisms that lack sophisticated genetics as well as for identifying components of existing biological pathways that are potential targets for drug discovery.

Text: Peptide-protein interactions have critical roles in biology. Many signals are transmitted by the binding of peptides to cell-surface receptors, and many protein-protein interactions inside cells are dominated by the binding of a peptide on one protein to a pocket on another. These interactions have inspired methods to select members of random peptide libraries that bind to known protein targets displayed on the outside of viruses (phage display) (B1) or within cells (B2) . An alternative strategy is to select peptides whose binding to unknown targets produces a phenotype in the same way that mutations produce phenotypes by inactivating genes (B3) . Like mutations, peptides can be used to probe the function and mechanism of biological pathways as well as to identify their in vivo protein targets.

We developed methods to express peptamers, peptides displayed as an exposed loop on the surface of an inert carrier protein, at high concentrations in budding yeast cells. This approach protects the peptides from proteolytic degradation and imposes some conformational rigidity (B4) . A catalytically inactive version of staphylococcal nuclease (B5) was used as a carrier protein because it is small, folds spontaneously without chaperones, has a prominently exposed loop on its surface (B6) , and can be strongly expressed as a soluble protein in eukaryotes and prokaryotes. The peptamer libraries contained 16 random amino acids inserted into the staphylococcal nuclease open reading frame (ORF) in place of the carrier's most exposed surface loop (B7) .

Because the extent of pathway inhibition depends on inhibitor concentration, we maximized expression of the peptamers. A high-copy vector was made that contains a strong constitutive promoter driving the expression of a staphylococcal nuclease gene that uses optimal codons for efficient translation and epitope tags for immunological detection and protein purification (B8) . Cells containing this vector expressed the peptamers as one of the most abundant proteins in the cell (Fig. 1).

We developed selections for inhibitors of two signal transduction pathways, the spindle checkpoint (B9) and the mating pheromone response pathway (B10) . The spindle checkpoint arrests cells in mitosis in response to chromosomes that fail to attach to the mitotic spindle (B11) and the pheromone pathway arrests cells in G.inf(1) in response to a peptide mating

factor. Both pathways are good targets for inhibitor selection because neither is essential for viability, and activation of either pathway prevents cell proliferation, creating a selection for peptamers that inhibit the pathway.

The spindle checkpoint is evolutionarily conserved and is defective in many human tumor cell lines (B12) . Selecting for inhibitors of the spindle checkpoint requires genetic trickery. In normal cells, the checkpoint is activated by improperly aligned chromosomes, and overriding the checkpoint in these cells leads to errors in chromosome segregation and cell death (B13) . However, overexpression of the checkpoint protein Mps1 activates the checkpoint in cells that have normal spindles (B14) . In this situation, inactivating the checkpoint allows cells to divide and form viable colonies. Thus, we engineered the selection strain to overexpress Mps1 when grown on galactose (B15) . We identified inhibitors of the spindle checkpoint by transforming the peptamer library into this strain and selecting for the rare transformants that formed colonies on galactose-containing medium (B16) . From a pool of 6.5 x 10.sup(6) transformants, we identified three peptamers that allow cells to proliferate on galactose (Fig. 2A). Two of the peptamers reduced the amount of the Mps1 protein (Fig. 2B). This effect appeared to be on the folding or stability of Mps1, because neither inhibitor reduced the amount of (beta) -galactosidase or another protein kinase (Cdc28) expressed from the same promoter (B17) .

We tested whether the putative checkpoint inhibitors could overcome the arrest caused by two other perturbations that activate the spindle checkpoint: depolymerization of mitotic spindle (Fig. 2C) and the presence of short linear minichromosomes (B17) . Only the inhibitor that did not alter the amount of Mps1 expression overcame the mitotic arrest caused by these other perturbations, which suggests that it alone is a general inhibitor of the spindle checkpoint.

The phenotypes of peptamers likely depend on binding to a protein target. We used the two-hybrid technique, which detects protein-protein interactions in yeast, as one approach to identify possible targets of inhibition (B18) . We fused the spindle checkpoint inhibitor to a DNA binding domain and tested it for interactions with a panel that expresses more than 85% of yeast genes fused to a transcriptional activation domain. In this assay, the inhibitor interacted strongly with an ORF of unknown function (YDR517W), which is the closest yeast homolog of GRASP65, a mammalian protein that is associated with the Golgi apparatus (B19) . Cells lacking Ydr517w have defects in the spindle checkpoint (Fig. 2D). We believe that the peptamer that binds to Ydr517w also interacts with other proteins because the peptamer overcomes the effects of Mps1 overexpression more strongly than the deletion of Ydr517w, even when the peptamer is expressed in ydr517w (Delta) cells. Green fluorescent protein fused to the COOH-terminus of Ydr517w produces punctate cytoplasmic fluorescence. Although the role of a cytoplasmic protein in the spindle checkpoint is not immediately obvious, our analysis shows that identifying proteins that peptamers bind to can uncover additional members of well-studied pathways.

We also isolated inhibitors of the pheromone response pathway. Budding yeast exist in two mating types, a and a, which can mate with each other only when both cells are in the G.inf(1) phase of the cell cycle. The a cells secrete a factor, which arrests a cells in G.inf(1) (Fig. 3A). Two types of peptamers allow a cells to form colonies on plates containing a factor: those that interfere with pheromone signaling directly and those that interfere with transcriptional silencing (B20) . The latter class cause haploid a cells to behave as pheromone-insensitive a/a diploids by allowing them to express a copy of the a genes (HMLa) that is present in a cells but is normally transcriptionally silent. Deleting HMLa restores a factor sensitivity in strains that carry silencing inhibitors but has no effect on the phenotype of signaling inhibitors.

We isolated 29 peptamers that allowed a cells to proliferate in the presence of a factor, of which 20 are silencing inhibitors (Table 1). Selecting for cell proliferation demands a minimum specificity of peptamers, because those that strongly inhibit essential processes are not recovered. To test the specificity of the silencing inhibitors more stringently, we performed a global analysis of peptamer effects on transcription. Using whole genome DNA microarray analysis, we compared the pattern of transcription of strains expressing two of the silencing inhibitors to that of a dominant-negative SIR4 mutant (SIR4.sup(DN)), which

disrupts repression at the silent mating-type loci and at telomeres. The transcriptional effects of the peptamers were highly correlated with those of SIR4.sup(DN) (Fig. 3B). Many MATa-specific genes (for example, STE2 and BAR1) and haploid-specific genes (for example, GPA1) showed correlated decreases in the abundance of their mRNAs, whereas the amount of MATa2 mRNA increased 1.5-to 2-fold, consistent with derepression of the silent loci. Furthermore, transcription of a number of genes located very near telomeres, including COS12, YER188W, and YAL069W, increased 1.6-to 2.5-fold, consistent with derepression of telomeric silencing. The absence of other widespread changes shows that the peptamers specifically inhibit silencing rather than causing general perturbations of cellular transcription.

We used the two-hybrid method to find proteins that interacted with five of the silencing inhibitors. Four of the five peptamers interacted strongly with specific proteins. One interacted with Asf1, whose overexpression or removal diminishes silencing (B21) . Another peptamer bound to Sfh1, an essential chromatin remodeling factor that interacts physically with components required for nucleosome restructuring (B22) .

Two silencing inhibitors that contain tryptophan-rich sequences (Table 1) interacted with two proteins, Tec1 and Dig1, and one of these peptamers also interacted weakly with Ste12. Both Tec1 and Dig1 interact with Ste12, a transcription factor required for the pheromone response; Dig1 was originally characterized as a negative regulator of Ste12 (B23) , and Tec1 interacts with Ste12 to stimulate pseudohyphal growth in diploids and invasive growth in haploids (B24) . Both peptamers blocked pseudohyphal growth in diploids, and one blocked invasive growth in haploids (B25) , phenotypes shared by both tec1 (Delta) and ste12 (Delta) mutants. The ability of these peptamers to inhibit silencing does not appear to depend on their interaction with Dig1, Tec1, or Ste12 because mating type silencing is maintained in a dig1 (Delta) tec1 (Delta) mutant and telomeric silencing is maintained in a ste12 mutant (B25) .

To characterize the nine pheromone signaling inhibitors, we used genetic tests to dissect the signaling pathway (Table 2). All the peptamers appeared to interfere downstream of Ste4, the G protein that interacts with the pheromone receptor, and upstream of Far1 the cyclin-dependent kinase inhibitor that directly induces cell cycle arrest (B26) . Five peptamers blocked a constitutively active allele of STE11(B27) , a mitogen-activated protein (MAP) kinase kinase kinase, from inducing transcription of a pheromone-responsive reporter gene, suggesting that they interfere with the MAP kinase cascade. We analyzed these inhibitors in more detail. The responses to pheromones and high osmolarity share a MAP kinase kinase kinase (Ste11) and certain other components (Ste20 and Ste50) but use different MAP kinases to produce their final output (B28) . A single peptamer increased the osmotic sensitivity of strains that depend on Ste11, Ste20, and Ste50 for osmotic signaling (Table 2), which suggests that this peptamer inhibits Ste11, Ste20, or Ste50. Two-hybrid experiments supported this conclusion: this peptamer interacted strongly with Ste50 and more weakly with Ste11, which binds to Ste50 (B29) . We believe that Ste50 is the target of the peptamer, because ste50 (Delta) cells show only modest a factor resistance, even in the presence of the peptamer, whereas expressing the peptamer in wild-type cells conferred strong resistance (Fig. 4). Other peptamers caused strong pheromone resistance in ste50 (Delta) , showing that these cells can become fully pheromone resistant. This result suggests that Ste50 has positive and negative functions in signaling and that the peptamer stimulates the activity of Ste50 that interferes with signaling, possibly by stimulating adaptation of the signaling pathway (B30) . One peptamer interacted with two MAP kinases (Fus3 and Kss1), either of which can transmit the pheromone signal, but failed to block the response to high osmolarity, which depends on the MAP kinase Hog1 (Table 2). This observation shows that peptamers are capable of discriminating between members of a family of homologous proteins.

Genetic selections can identify peptamers that inhibit biological pathways. Both of our selections produced two classes of peptamers, those that interfered indirectly with the target pathway and those that did so directly. Thus we obtained inhibitors of Mps1 expression as well as those that directly inhibit the spindle checkpoint and inhibitors of transcriptional silencing and those that interfere directly with pheromone signaling. These results highlight the importance of secondary tests that provide independent information about how each inhibitor passed the

original selection.

We used three different methods to gain information about the targets of the peptamers: two-hybrid analysis, transcript arrays, and pathway dissection. Each approach has strengths and weaknesses, and a combination of methods is likely to be the most reliable way to identify targets. Two-hybrid analysis is simple, reports on physical interactions, and can identify additional components of existing pathways. The major drawbacks are false positives and false negatives: 7 of the 15 peptamers we tested failed to give a reproducible interaction with any protein, 3 peptamers showed interactions with more than one protein, and, in two cases, genetic tests suggested that none of the interacting proteins was the physiological target of the peptamer. Analyzing transcript arrays tests whether peptamers produce a similar response to known genetic perturbations and is the most powerful way of analyzing the specificity of inhibition. However, if mutations in multiple genes produce a similar transcriptional response, the product of any one of these genes could be the target of a peptamer. Genetic tools that dissect a pathway into separate modules are good reagents for directly identifying the targets of peptamers, but they are available for only a small number of well-studied pathways. Other methods that may identify targets include screening for proteins whose overexpression overcomes the inhibitory phenotype and mass spectrometry-based identification of proteins that bind to peptamers.

Genetic selection of inhibitory peptamers has four possible applications to drug discovery. First, by selecting for proliferation of the cells in which the target pathway has been inhibited, it provides a useful minimum of specificity. Second, it identifies new targets for drug discovery by finding new components of the target pathway. Third, a protein whose function can be inhibited with a short peptide is likely to be a good candidate for inhibition by small organic molecules. Finally, genetic selections make it easy to isolate peptamers with higher and lower potencies, and correlations between structure and activity could be used to guide combinatorial or peptidomimetic chemistry.

Figure F1
Caption: Peptamer expression in yeast. Measurement of peptamer expression in yeast by Coomassie staining (left) and antihemagglutinin immunoblotting (right). Double-headed arrow denotes staphylococcal nuclease; double-headed closed circle denotes peptamer P-7. Numbers on left are kilodaltons.

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Figure F2
Caption: Identification of an inhibitor of the spindle checkpoint. (A) Effects of three peptamers on the spindle assembly checkpoint activated by overexpression of Mps1. Cultures of logarithmic-phase controls and inhibitor-containing strains (B15) were equalized for cell density, serially diluted by a factor of three, spotted onto -uracil/+galactose plates, and incubated at 30.Deg.C. GAL-MPS1 resistance was also measured by plating equal numbers of logarithmic-phase cells onto -uracil/+glucose and -uracil/+galactose plates and incubating at 30.Deg.C for 4 days. CDC20-127 is a dominant mutant that inactivates the spindle checkpoint (B33) and serves as a positive control. Percent resistance is defined as 100 times the number of colonies on the -uracil/+galactose plate divided by the number of colonies growing on the -uracil/+glucose plate. (B) Reduced amounts of Mps1 expression in peptamer-containing strains. To avoid cell-cycle-regulated changes in the stability of Mps1, we arrested all cells in mitosis with the cdc23-1 mutant, which lacks anaphase-promoting complex activity at high temperatures. Haploid strains (GAL-MPS1::URA3, cdc23-1) containing control or inhibitory peptamers were grown at 23.Deg.C in -uracil/+raffinose to early logarithmic phase and then shifted to 34.Deg.C in -uracil/+galactose for 4 hours to induce transcription of Mps1. Extracts were prepared and analyzed by protein immunoblotting with antibodies to Myc epitope-tagged Mps1. Numbers on left are kilodaltons. (C) Spindle checkpoint inhibition by peptamer C-3. Exponentially growing cells containing a control peptamer or inhibitor C-3 were arrested in G.inf(1) by treatment with a factor for 4 hours. After a factor was washed out, cells were (i) sonicated briefly, incubated on -uracil/+benomyl (20 (mu) g/ml) plates, and scored for the percentage of cells that rebud after 5 hours at 16.Deg.C; or (ii) treated with nocodazole (15 (mu) g/ml) for 0, 2, 3.3, and 5 hours and plated for viability. (Delta) , Staphylococcal nuclease;

x , CDC20-127; (square-solid) , peptamer C-3. (D) Effect of deleting the YDR517w gene on the spindle checkpoint. Wild-type (WT) cells, wild-type cells plus a control peptamer or peptamer C-3 (which binds Ydr517w), and YDR517w (Delta) cells were tested for their ability to rebud in the presence of benomyl as in (C).

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Figure F3

Caption: Identification of a factor-resistent peptamers that inhibit transcriptional silencing. (A) Cartoon of the pheromone response pathway in budding yeast. (B) Comparisons of the transcript array profiles of two silencing inhibitors and a dominant-negative mutant of the silencing protein Sir4. RNA samples were prepared from wild-type cells and from cells expressing peptamer S-1, S-5, or a dominant-negative allele of SIR4 and were analyzed by competitive hybridization to DNA microarrays containing >6200 yeast ORFs (B34) . Correlation plots comparing the results of these hybridizations are shown (B35) . (Top) Log.inf(10)of the expression ratio of ORFs from cells containing or lacking peptamer S-1 [(log.inf(10)(R/G)] plotted versus the log.inf(10) ratio of ORFs from cells containing or lacking peptamer S-5 expression. (Bottom) Log.inf(10)(R/G) ratios of ORFs from cells containing or lacking peptamer S-1 expression plotted versus ORFs from cells with or without SIR4.sup(DN) expression. For each plot, >5600 ORFs (91 to 96% of total spots) for which reliable data were measured are plotted. ORFs whose expression did not change in either experiment are plotted as gray dots; ORFs that changed significantly (P <= 0.01) in expression in both experiments are plotted as red stars; ORFs that changed significantly (P <= 0.01) only in the competitive hybridization plotted on the x or y axis are plotted as green or blue stars, respectively. Specific subtelomeric, MATa-, and haploid-specific genes are labeled. Supplementary material is available at: www.sciencemag.org/feature/data/1041079.shl

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Begin Table : Columns 1 - 5 of 5

Caption:
Amino acid sequence Reference B31 and characterization of 20 silencing inhibitors. NA, not applicable; NI, no interaction detected; NT, not tested.

Gene on plasmid	Amino acid sequence	a-Factor Resistance (%)	Induces sporulation	Two-hybrid interaction
Staph nuclease	-	0	No	NA
MATa	-	100	Yes	NA
SIR4DN	-	100	Yes	NA
Inhibitor S-1 (7 aa)	VCLGGVP	82	Yes	NT
Inhibitor S-2 (15 aa)	RFFWNPWTRVMQRAP	66	Yes	NT
Inhibitor S-3 (4 aa)	WVNW	43	No	NT
Inhibitor S-4 (15 aa)	RRTGGWGGNTCIIKFD	9	No	NT
Inhibitor S-5 (4 aa)	WVGW	70	No	DIG1, TEC1, STE12
Inhibitor S-6	VYLRKFSKVVPITWGW	59	No	NT
Inhibitor S-7 (9 aa)	VVWLDCW	100	Yes	DIG1, TEC1
Inhibitor S-8 (6 aa)	GRMEPGAAPRDSKCNA	49	No	NT
Inhibitor S-9	SLLATRSAKLALCSAR	91	Yes	NT
Inhibitor S-10	ILIKSKMHQRTLFSAL	100	Yes	SFH1
Inhibitor S-11	VYWRGQSLYATLSTSE	94	Yes	ASF1
Inhibitor S-12	VPSLRALWAYAGLGDS	79	Yes	NT
Inhibitor S-13	PCLVSSGPAGRSPSAW	11	Yes	NT
Inhibitor S-14	VYRCGPGGVLYPPACR	72	No	NT
Inhibitor S-15	PLLDPQQHAAPVAAGP	92	No	NI
Inhibitor S-16	ILLTRVHLRRSYMGAT	18	Yes	NT

Inhibitor S-17	FVFARRGYHLASTVHT	59		No		NT
Inhibitor S-18	CVACGLKLAGRLVGYL	85		No		NT
Inhibitor S-19	LLWSSVVKNPKFGGLF	100		No		NT
Inhibitor S-20	RLMSWRDSLWSYARLS	83		Yes		NT

Footnote:
Equal numbers of logarithmic-phase cells were plated on -uracil/+glucose plates either lacking or containing a factor (1 (mu) g/ml) and incubated at 30.Deg.C for 3 days. Percent resistance is 100 times the number of colonies on medium with a factor divided by the number of colonies growing on the plate lacking a factor.
Footnote:
A MATa/MATa strain was transformed with control or inhibitory peptamers, grown to saturation in -uracil/+glucose, and transferred to sporulation medium (2% KOAc, 0.02% raffinose) and incubated at 23.Deg.C for 3 days. Cells were analyzed under a microscope for the presence of tetrads.
Footnote:
Five peptamers were fused to a DNA binding domain and tested for two-hybrid interactions against a panel containing >85% of the ORFs in yeast fused to a transcriptional activation domain Reference B32 .
End Table: Columns 1 - 5 of 5

Begin Table : Columns 1 - 7 of 8

Caption:
Amino acid sequence and characterization of nine pheromone signaling inhibitors.

Gene on plasmid	Amino acid sequence	a-factor Resistance (%)	At or below Ste4	From Ste11 to Fus1	At Ste11, Ste20, or Ste50	At or below Far1
Staph nuclease	-	0	NA	NA	NA	NA
MATa	-	100	NA	NA	NA	NA
SIR4DN	-	100	NA	NA	NA	NA
Inhibitor P-1	LYATRGLVRSHVCLGL	44	Yed	Yes	No	No
Inhibitor P-2	LLWSSVVKNPKFRHLF	100	Yes	No	No	No
Inhibitor P-3	WWVRREIWFGAVISYE	42	?	No	No	No
Inhibitor P-4	CRSVKEALVVFRRMLQ	100	Yes	Yes	No	No
Inhibitor P-5	RIKGRYLAFVRQVGGF	51	Yes	No	No	No
Inhibitor P-6	CWVCVPRVLRQRLLGI	76	Yes	No	No	No
Inhibitor P-7	VLDVKDASDESILLSW	100	Yes	Yes	Yes	No
Inhibitor P-8	HGGVPGRPPSFILWKM	75	Yes	Yes	No	No
Inhibitor P-9	EIRRWVQATYPLFASS	76	Yes	Yes	No	No

Footnote:
See Table 1<TBLR RID="T1"> for determination of percent a factor resistance.
Footnote:
Ability of peptamers to overcome the cell-cycle arrest generated by galactose-induced overexpression of Ste4 (pGAL-STE4).
Footnote:
Ability of peptamers to overcome the signaling of a constitutively active STE11-1 mutant. Cells containing a FUS1-HIS3 reporter induced by STE11-1 were transformed with control or inhibitory peptamers and tested for their ability to grow on -uracil/-histidine plates.
Footnote:
Osmotic sensitivity of a strain that depends on Ste11, Ste20 and Ste50 for osmotic signaling. A haploid MATa ssk1 strain Reference B28 that uses the Sho1 osmosensor and Ste11, Ste20, and Ste50 for osmoresistance was transformed with control or inhibitory peptamers. Transformants were tested for osmosensitivity by streaking onto -uracil/0.9 M NaCl plates.
Footnote:
Ability of peptamers to overcome the cell-cycle arrest generated by galactose-induced overexpression of a dominant FAR1-22 mutant.
Footnote:
See legend to Table 1<TBLR RID="T1">.

End Table: Columns 1 - 7 of 8
Begin Table : Columns 8 - 8 of 8

Caption:
Amino acid sequence and characterization of nine pheromone signaling inhibitors.

Gene on plasmid	Two-hybrid interaction
Staph nuclease	NA
MATa	NA
SIR4DN	NA
Inhibitor P-1	FUS3, KSS1
Inhibitor P-2	NI
Inhibitor P-3	NT
Inhibitor P-4	NI
Inhibitor P-5	NT
Inhibitor P-6	NI
Inhibitor P-7	STE11, STE50
Inhibitor P-8	NI
Inhibitor P-9	ARG80

Footnote:
See Table 1<TBLR RID="T1"> for determination of percent a factor resistance.
Footnote:
Ability of peptamers to overcome the cell-cycle arrest generated by galactose-induced overexpression of Ste4 (pGAL-STE4).
Footnote:
Ability of peptamers to overcome the signaling of a constitutively active STE11-1 mutant. Cells containing a FUS1-HIS3 reporter induced by STE11-1 were transformed with control or inhibitory peptamers and tested for their ability to grow on -uracil/-histidine plates.
Footnote:
Osmotic sensitivity of a strain that depends on Ste11, Ste20 and Ste50 for osmotic signaling. A haploid MATa ssk1 strain Reference B28 that uses the Sho1 osmosensor and Ste11, Ste20, and Ste50 for osmoresistance was transformed with control or inhibitory peptamers. Transformants were tested for osmosensitivity by streaking onto -uracil/0.9 M NaCl plates.
Footnote:
Ability of peptamers to overcome the cell-cycle arrest generated by galactose-induced overexpression of a dominant FAR1-22 mutant.
Footnote:
See legend to Table 1<TBLR RID="T1">.
End Table: Columns 8 - 8 of 8

Figure F4
Caption: Genetic identification of a peptamer target. Cultures of logarithmic-phase MATa, ste11 (Delta) MATa, or ste50 (Delta) MATa cells expressing staphylococcal nuclease, peptamer P-7, or peptamer S-7 were equalized for cell density, serially diluted by a factor of five, and spotted onto plates containing yeast extract, peptone, and dextrose (YPD) and a factor (1 (mu) g/ml) and incubated at 30.Deg.C for 3 days.
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7. The library in Escherichia coli contained 1.7 x 10.sup(8) members, of which more than 1.14 x 10.sup(8) directed the synthesis of peptamers. The synthetic **staphylococcal** nuclease gene was assembled in two cloning steps: 15 oligonucleotides were annealed and ligated to form double-stranded NH.inf(2)-terminal (177 bases) and COOH-terminal (330 bases) fragments that were ligated separately to vector (pTCN13) cut with Bst EII and Eco RI and with Eco RI and Sal I, respectively. The Eco RI and Sal I sites allow insertion of a loop that replaces amino acids 19 to 27 of the mature **staphylococcal** nuclease. The resulting constructs were digested with Eco RI and Xba I and ligation of the **staphylococcal** nuclease gene-containing fragments assembled the full ORF fused to a hemagglutinin epitope tag at the NH.inf(2)-terminus and to a stretch of six histidine residues (pTCN22) at the COOH-terminus. The random peptide insert was prepared by self-annealing the following oligonucleotide: 5'-CCCGAATTCTTCGGTGGT(NNS).inf(16)GGTGGTGTCGACAC- **3** ' [N = A:T:G:C (1:1:1:1), S = G:C (1:1)]. Annealed DNA was extended by using the Klenow fragment of DNA polymerase I, and the double-stranded product was cut with Eco RI and Sal I to create two equivalents of library DNA that were ligated to the **staphylococcal** nuclease construct (pTCN22) cut with Eco RI and Sal I, installing library DNA in-frame. ;
8. The expression vector pTCN23 carried the replicator of the 2- (mu) m circle, an endogenous yeast high-copy plasmid, the PGK1 promoter [A. Chambers, C. Stanway, A. J. Kingsman, S. M. Kingsman, Nucleic Acids Res. 16, 8245 (1988)], and had codon usage optimized according to Sharp and Li [P. M. Sharp and W. H. Li, Nucleic Acids Res.15, 1281 (1987). ;
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16. For both selections, transformants were grown on uracil-deficient plates for 2 days. Cells were scraped from these plates, and aliquots were plated onto selection plates [lacking uracil and containing galactose (-uracil/+galactose) for the spindle checkpoint selection; lacking uracil and containing a factor (-uracil/+a factor) (1 (mu) g/ml) for the pheromone pathway selection] and grown at 30.Deg.C for 2 or **3** days. Plasmid DNA was recovered from the entire population of colonies that grew on these plates, purified, amplified by transformation into E. coli, and retransformed into DA2050A to enrich for peptamers that conferred resistance as opposed to genomic mutations that caused resistance. Cell populations harboring active peptamers typically grew as lawns on selection plates, and individual plasmids were isolated from these plates and retested to confirm that they gave plasmid-dependent resistance. ;
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31. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. ;
32. A yeast ORF-Gal4 activation domain fusion array has been assembled that expresses about 85 to 90% of the predicted ORFs of S. cerevisiae in strain pJ69-4a [P. James, J. Halladay, E. Craig Genetics 144, 1425 (1996)]. In this strain, GAL4 is absent and the reporters HIS3 and ADE2 are under Gal4 control. To probe the Gal4-AD fusion array for protein-protein interactions, we mated the array to a strain expressing a Gal-4-DNA binding domain fusion. After selecting for diploids, we identified two-hybrid positives by testing diploids on plates containing different concentrations of 3 -aminotriazole. ;
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26/9/36 (Item 36 from file: 71)

DIALOG(R)File 71:ELSEVIER BIOBASE

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Identification of genes encoding two-component lantibiotic production in Staphylococcus aureus C55 and other phage group II S. aureus strains and demonstration of an association with the exfoliative toxin B gene

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The production of exfoliative toxin B (ET-B), but not ET-A, was shown to be specifically associated with production of a highly conserved two-component lantibiotic peptide system in **phage** group II **Staphylococcus aureus** . Two previously studied but incompletely characterized S. **aureus** bacteriocins, **staphylococcins** C55 and BacR1, were found to be members of this **antibiotic** system, and considerable homology was also found with the two-component Lactococcus lactis bacteriocin, lacticin 3147. sacalphaA and sacbetaA, the structural genes of the lantibiotics **staphylococcins** C55alpha and C55beta and two putative lantibiotic processing genes, sacM1 and sacT, were localized together with the ET-B structural gene to a single 32-kb plasmid in strain C55. Irreversible loss of both ET-B and two-component lantibiotic production occurs during laboratory passage of ET-B-positive S. **aureus** strains, particularly at elevated temperatures.

SPECIES DESCRIPTORS:
Staphylococcus aureus

CLASSIFICATION CODE AND DESCRIPTION:
84. **3** .7 - GENETICS AND MOLECULAR BIOLOGY / PROKARYOTIC GENETICS / Genetics of Animal Pathogenesis
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26/9/48 (Item 48 from file: 73)
DIALOG(R)File 73:EMBASE

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96th General meeting of the American Society for Microbiology
Goldschmidt R.M.; Frosco M.; Alaparthy S.; Barrett J.F.
RW Johnson Pharmaceutical Res. Inst.,Raritan, NJ 08869 United States
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The 96th Annual Meeting of the American Society for Microbiology covered an eclectic blend of presentations including: old therapeutic drugs looking for new recommendations, new drugs in the process of approval or in various phases of clinical trials, new developments in bacterial pathogenesis, protein secretion and immunomodulation.

BRAND NAME/MANUFACTURER NAME: augmentin/smith kline beecham/United States; unasyn/pfizer; ly 264826
MANUFACTURER NAMES: nexstar/United States; smith kline beecham/United States; pfizer
DRUG DESCRIPTORS:
*antiinfective agent--drug therapy--dt; *beta lactam **antibiotic** --drug therapy--dt; *beta lactam **antibiotic** --clinical trial--ct; *beta lactam **antibiotic** --drug dose--do; *cytokine; *glycopeptide--drug development--dv; *macrolide; *quinoline derived antiinfective agent--drug therapy--dt; * quinoline derived antiinfective agent--drug dose--do; *quinoline derived antiinfective agent--drug administration--ad
amikacin; amoxicillin plus clavulanic acid--drug therapy--dt; amoxicillin plus clavulanic acid--drug dose--do; amoxicillin plus clavulanic acid --clinical trial--ct; azithromycin; beta lactamase; cefoxitin; chloroorienticin a--drug development--dv; ciprofloxacin--pharmacokinetics --pk; ciprofloxacin--drug comparison--cm; ciprofloxacin--drug therapy--dt; erythromycin; gamma interferon--drug development--dv; granulocyte colony stimulating factor--drug development--dv; granulocyte colony stimulating factor--drug dose--do; immunomodulating agent; interleukin 12--drug development--dv; interleukin 13--drug development--dv; levofloxacin--drug administration--ad; levofloxacin--drug dose--do; levofloxacin--drug therapy

--dt; levofloxacin--pharmacokinetics--pk; levofloxacin--drug comparison--cm ; liposome; metenkephalin; new drug; protamine; sultamicillin; teicoplanin; trovafloxacin--clinical trial--ct; trovafloxacin--drug dose--do; trovafloxacin--drug therapy--dt; unindexed drug; vancomycin--drug concentration--cr; zidovudine
MEDICAL DESCRIPTORS:
*bacterial infection--drug therapy--dt; *mycobacteriosis--drug therapy--dt; *mycosis--drug therapy--dt
animal experiment; animal model; **antibiotic** resistance; **bacteriophage** ; bone infection--drug therapy--dt; clinical trial; conference paper; controlled study; dose response; double blind procedure; drug half life; drug screening; enterococcus; human; immunomodulation; intramuscular drug administration; intraperitoneal drug administration; minimum **inhibitory** concentration; mouse; multicenter study; nonhuman; phase **3** clinical trial ; protein secretion; pseudomonas aeruginosa; randomized controlled trial; skin infection--drug therapy--dt; **staphylococcus** ; subcutaneous drug administration
CAS REGISTRY NO.: 37517-28-5, 39831-55-5 (amikacin); 74469-00-4 (amoxicillin plus clavulanic acid); 83905-01-5 (azithromycin); 9073-60-3 (beta lactamase); 33564-30-6, 35607-66-0 (cefoxitin); 118395-73-6 (chloroorienticin a); 85721-33-1 (ciprofloxacin); 114-07-8, 70536-18-4 (erythromycin); 82115-62-6 (gamma interferon); 138415-13-1 (interleukin 12); 148157-34-0 (interleukin 13); 100986-85-4, 138199-71-0 (levofloxacin); 58569-55-4 (metenkephalin); 11061-43-1, 9007-31-2, 9012-00-4 (protamine); 76497-13-7 (sultamicillin); 61036-62-2, 61036-64-4 (teicoplanin); 146836-84-2 (trovafloxacin); 1404-90-6, 1404-93-9 (vancomycin); 30516-87-1 (zidovudine)
SECTION HEADINGS:
030 Clinical and Experimental Pharmacology
037 Drug Literature Index

26/9/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10031322 99028371 PMID: 9810676
Characteristics of Staphylococcus aureus strains isolated from clinical and non-clinical human sources in Trinidad: susceptibility to bacteriophages and antimicrobial agents, and toxigenicity.
Adesiyun A A; Prabhakar P; Ali C; Lewis M
Faculty of Medical Sciences, University of the West Indies, St. Augustine, Trinidad.
Zentralblatt fur Bakteriologie : international journal of medical microbiology (GERMANY) Oct 1995, 282 (4) p519-32, ISSN 0934-8840
Journal Code: 9203851
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The susceptibility of Staphylococcus aureus strains isolated from human clinical and non-clinical sources in Trinidad to bacteriophages and antimicrobial agents was determined. The ability of the strains to produce enterotoxins and toxic shock syndrome toxin-1 (TSST-1) was also investigated. Of the 554 strains tested, 454 (81.8%) were susceptible to international phage set (IPS) **phages** with strains isolated from bacteruria (57.1%) and bacteremia (53. **3** %) having a low sensitivity compared to isolates from aspirates (87. **3** %) and anterior nares (97.4%). All sources combined, strains were most susceptible to **phages** belonging to several groups (mixed). Overall, 419 (75.6%) strains were resistant to one or more of nine **antimicrobial** agents tested. Resistance to penicillin was most prevalent, with 413 (74.5%) strains found to be resistant. Prevalence of resistance to tetracycline, gentamicin, oxacillin, cefuroxime and ciprofloxacin was 5.1%, 2.0%, 0.7%, 0.4% and 0.4%, respectively. Of the 554 strains tested, 307 (55.4%) produced **staphylococcal** enterotoxins A (SEA), B (SEB), C (SEC) and D (SED) singly or in combination. Strains recovered from high vaginal swabs were least enterotoxigenic (40.0%) as compared to umbilical infection isolates which were most enterotoxigenic (78.9%). TSST-1 was produced by 95 (19.0%) out of 499 strains tested, with

isolates from bacteruria found to be most toxigenic (33. 3 %). It was concluded that the S. **aureus** strains tested were highly susceptible to **bacteriophages** and **antimicrobial** agents (except penicillin) and that enterotoxigenic and TSST-1 producers were widespread and have an aetiologic potential.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: *Antibiotics--pharmacology--PD; *Bacteriophages--physiology --PH; *Enterotoxins--biosynthesis--BI; *Staphylococcus aureus; *Staphylococcus aureus--metabolism--ME; Staphylococcal Infections --microbiology--MI; Staphylococcus aureus--drug effects--DE; Staphylococcu s aureus--virology--VI; Trinidad and Tobago

CAS Registry No.: 0 (Antibiotics); 0 (Enterotoxins); 0 (enterotoxin F, Staphylococcal); 12788-99-7 (enterotoxin D, Staphylococcal); 37337-57-8 (enterotoxin A, Staphylococcal); 39424-53-8 (enterotoxin B, staphylococcal); 39424-54-9 (enterotoxin C, staphylococcal)

Record Date Created: 19990122

26/9/14 (Item 14 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

10792597 20323749 PMID: 10865428

[Evaluation of the usefulness of new international experimental phages for typing methicillin resistant Staphylococcus aureus (MRSA)]

Ocena przydatnosci nowego, miedzynarodowego zestawu eksperymentalnych fagow do typowania opornych na metycyline gronkowcow zlocistych (MRSA).

Piechowicz L; Wisniewska K; Galinski J

Medycyna doswiadczalna i mikrobiologia (POLAND) 1999, 51 (1-2) p31-6 , ISSN 0025-8601 Journal Code: 0210575

Document type: Journal Article ; English Abstract

Languages: POLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The aim of the study was to determine the usefulness of the set of experimental phages obtained from the Central Public Health Laboratory in London for typing of MRSA strains in Poland. The study was performed on 150 MRSA strains isolated from various clinical materials in various regions of the country. The set of 10 experimental phages and the international basic set of 23 phages were used for typing. The results of the study showed that 76.8% of MRSA strains were typing with the experimental set of **phages** . The frequency of **inhibition** reactions was 19.9%. Only 3 . 3 % of the strains were nontypable with the new **phages** while nearly half of the studied strains were nontypable with the basic set of **phages** . The studied strains were divided into 19 phagotypes. There was a high frequency of typable strains among MRSA typable and nontypable strains and those **inhibited** by the basic set of **phages** (71.4%-85.7%). These data indicate that the set of 10 experimental **phages** is useful for typing of MRSA strains isolated in Poland except for **phage** M3 which failed to react with almost all the strains and should be excluded from the proposed set.

Descriptors: *Bacteriophage Typing--methods--MT; *Bacteriophages --classification--CL; *Methicillin Resistance; *Staphylococcus aureus --classification--CL; *Staphylococcus aureus--drug effects--DE; Species Specificity

Record Date Created: 20000925

26/9/39 (Item 39 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

10053779 99028817 PMID: 9812283

Protein antimicrobial barriers to bacterial adhesion.

Bower C K; Daeschel M A; McGuire J

Department of Bioresource Engineering, Oregon State University, Corvallis, USA.

Journal of dairy science (UNITED STATES) Oct 1998, 81 (10) p2771-8, ISSN 0022-0302 Journal Code: 2985126R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The ability of microorganisms to adhere to solid surfaces is a problem of high visibility and has been the focus of numerous investigations because these organisms can cause disease and food spoilage. During the last several years, considerable attention has been focused on the development of food-grade **antimicrobial** barriers to adhesion in order to **inhibit** the initial adhesion of microbial contaminants by application of an **antimicrobial** agent to the surface rather than trying to remove these contaminants once they are adhered. The premise is that, if both the presence of the agent and its **antimicrobial** activity are maintained at the interface, sensitive bacterial cells or spores that attempt to attach would be killed. Nisin has been used in foods as a direct additive to **inhibit** the growth of Gram-positive cells and spores. Similarly, hen lysozyme is a commercially available **antimicrobial** protein that offers application in food processing systems, but the mode of action of this enzyme differs from that of nisin. We have shown that nisin can adsorb to surfaces, maintain activity, and kill cells that have adhered. In addition, we have addressed questions relating to the short- and long-term stability of adsorbed nisin, the degree to which immobilized nisin can resist exchange with dissolved solution components, and the surface concentrations that are necessary to **inhibit** biofilm formation. More recently, we have focused on basic questions relating to molecular influences on **antimicrobial** activity at interfaces using synthetic mutants of **bacteriophage** T4 lysozyme and hen lysozyme in addition to nisin.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.

Descriptors: *Anti-Infective Agents; *Bacterial Adhesion; *Biofilms; *Food Handling--methods--MT; *Proteins; Adsorption; Antibiotics, Peptide; Bacteriophage T4--enzymology--EN; Circular Dichroism; Enterococcus faecalis --physiology--PH; Listeria monocytogenes--physiology--PH; Muramidase --chemistry--CH; Nisin--chemistry--CH; Proteins--chemistry--CH; Staphylococ cus--physiology--PH

CAS Registry No.: 0 (Anti-Infective Agents); 0 (Antibiotics, Peptide) ; 0 (Proteins); 1414-45-5 (Nisin)

Enzyme No.: EC 3 .2.1.17 (Muramidase)

Record Date Created: 19990119

04198178 Genuine Article#: RM672 Number of References: 41
Title: HETEROGENEOUS ENDOLYSINS IN LISTERIA-MONOCYTOGENES BACTERIOPHAGES - A NEW CLASS OF ENZYMES AND EVIDENCE FOR CONSERVED HOLIN GENES WITHIN THE SIPHOVIRAL LYSIS CASSETTES
Author(s): LOESSNER MJ; WENDLINGER G; SCHERER S
Corporate Source: TECH UNIV MUNICH,FORSCHUNGSZENTRUM MILCH & LEBENSMITTEL WEIHENSTEP,INST MIKROBIOL/D-85350 FREISING//GERMANY/
Journal: MOLECULAR MICROBIOLOGY, 1995, V16, N6 (JUN), P1231-1241
ISSN: 0950-382X
Language: ENGLISH Document Type: ARTICLE
Geographic Location: GERMANY
Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY; MICROBIOLOGY
Abstract: Listeria monocytogenes bacteriophages A118, A500 and A511 are members of three distinct phage groups with characteristic host ranges. Their endolysin (ply) genes were cloned and expressed in Escherichia coli as demonstrated by the conferred lytic phenotype when colonies of recombinant cells were overlaid with a lawn of Listeria cells. The nucleotide sequences of the cloned DNA fragments were determined and the individual enzymes (PLY118, 30.8 kDa; PLY500, 33.4 kDa; PLY511, 36.5 kDa) were shown to have varying degrees of homology within their N-terminal or C-terminal domains. Transcriptional analysis revealed them to be 'late' genes with transcription beginning 15-20 min post-infection. The enzymes were overexpressed and partially purified and their individual specificities examined. When applied exogenously, the lysins induced rapid lysis of Listeria strains from all species but generally did not affect other bacteria. Using hydrolysis of purified listerial cell walls, PLY511 was characterized as an N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28) and shows homology in its N-terminal domain to other enzymes of this type. In contrast, PLY118 and PLY500 were shown to represent a new class of cell wall lytic enzymes which cleave between the L-alanine and D-glutamate residues of listerial peptidoglycan; these were designated as L-alanoyl-D-glutamate peptidases. These two enzymes share homology in the N-terminal domain which we propose determines hydrolytic specificity. Highly conserved **holin** (hol) gene sequences are present upstream of ply118 and ply500. They encode proteins of structural similarity to the product of phage lambda gene S, and are predicted to be membrane proteins which form pores to allow access of the lysins to their peptidoglycan substrates. This arrangement of conserved **holin** genes with downstream lysin genes among the siphoviral lysis cassettes explains why the cytoplasmic endolysins alone are not lethal, since they require a specific transport function across the cell membrane.
Identifiers--KeyWords Plus: LAMBDA-S-PROTEIN; RNA- **POLYMERASE** ; PHAGE PHI-29; SEQUENCE-ANALYSIS; ESCHERICHIA-COLI; ENCODING GENE; CLONED GENES; EXPRESSION; CLONING; BACTERIA
Research Fronts: 93-8060 002 (MEMBRANE DOMAIN OF A BACTERIOPHAGE ASSEMBLY PROTEIN; ESCHERICHIA-COLI K-12; SYNTHETIC GENE; AFRICAN SWINE FEVER VIRUS; PHI-29 DNA- **POLYMERASE** ACTIVE-SITE)
93-2115 001 (FERMENTATION OF THE PRODUCING STRAIN; PRADIMICIN DERIVATIVES; ANTIFUNGAL ACTIVITY; DEVELOPMENTAL THERAPEUTICS; CORYNEFORM BACTERIA)
93-3981 001 (LISTERIA-MONOCYTOGENES SURVIVAL IN WHITE PICKLED CHEESE; COLORIMETRIC NUCLEIC-ACID HYBRIDIZATION ASSAY; ENRICHED CULTURES OF INOCULATED FOODS)
93-4847 001 (HETEROLOGOUS EXPRESSION; CHROMOSOMAL DNA; GENE ENCODING METHYLMALONYL-COENZYME-A MUTASE)
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32/9/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09814013 98232107 PMID: 9572396
The lambda holin **accumulates beyond the lethal triggering concentration under hyperexpression conditions.**
Smith D L; Chang C Y; Young R Y
Department of Biochemistry and Biophysics, Texas A&M University, College Station 77843, USA.
Gene expression (UNITED STATES) 1998, 7 (1) p39-52, ISSN 1052-2166 Journal Code: 9200651
Contract/Grant No.: GM27099; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Most bacteriophages terminate infection by creating lesions in the cytoplasmic membrane, which not only cause immediate cell death but also allow escape of a phage-encoded endolysin. Destruction of the peptidoglycan and cell lysis follows very rapidly, allowing efficient release of the progeny virions. These membrane lesions are formed by a small integral membrane protein called a **holin** . **Holins** have highly charged carboxyl-termini that are thought to have two transmembrane alpha-helical domains. **Holins** are believed to oligomerize and form large holes in the inner membrane. The prototype **holin** is the S protein from bacteriophage lambda. Scheduling of the lytic event is determined in part by the "structure directed initiation" or sdi translational control region. Inductions of S, cloned under a variety of native and nonnative promoters but with native translational control, resulted in cell lysis at about 1000 molecules of **holin** per cell, and thus do not produce biochemically useful amounts of S protein. By utilizing a plasmid-based system with the T7 RNA **polymerase** promoter in tandem with a consensus ribosome binding site, Coomassie blue-detectable quantities of S protein were obtained upon

03751703 H.W. WILSON RECORD NUMBER: BGSI98001703 (THIS IS THE FULLTEXT)
Antisense RNA-regulated programmed cell death.
Gerdes, Kenn
Gultyaev, Alexander P; Franch, Thomas
Annual Review of Genetics (Annu Rev Genet) v. 31 ('97) p. 1-31
SPECIAL FEATURES: bibl il ISSN: 0066-4197
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
RECORD TYPE: Abstract; Fulltext RECORD STATUS: Corrected or revised record
WORD COUNT: 12418

ABSTRACT: The characteristics of the hok gene family were examined. These plasmid-encoded killer genes are responsible for plasmid stabilization by killing plasmid-free cells. The details of the complicated antisense RNA-regulated control loop that controls posttranscriptional and postsegregational activation of killer mRNA translation in plasmid-free cells are discussed. Nucleotide covariations in the mRNAs identify metastable stem-loop structures that form at the mRNA 5' end in the nascent transcripts and prevent translation and antisense RNA binding during transcription. Coupled nucleotide covariations are evidence of a phylogenetically conserved mRNA folding pathway that involves sequential dynamic RNA rearrangements. Thus, there is an intricate mechanism that allows for the conditional activation of translation of an antisense RNA-controlled mRNA. The complex phylogenetic relationships of the plasmid- and chromosome-encoded systems are also described.

TEXT:

KEY WORDS
hok, sok, RNA rearrangements, RNA folding pathway, cell killing

INTRODUCTION

Bacterial plasmids are inherited independently of the host cell chromosome. Despite their extrachromosomal status, bacterial plasmids are stably maintained. The stable maintenance may be due to a high copy number of the elements. However, in low-copy-number plasmids, stable inheritance depends on the presence of a number of gene systems that, by different mechanisms, actively prevent the appearance of plasmid-free progeny (28, 38, 61). Unitcopy plasmids such as F, P1, and R1 encode centromere-like functions that are thought to equi-partition the plasmid molecules at cell division (11, 61). The group of proteic plasmid stabilization systems constitutes a second type of maintenance systems (42). These latter systems mediate plasmid stabilization by killing plasmid-free segregants. The proteic systems specify two protein components: a stable toxin and an unstable antidote. The antidotes neutralize their cognate toxins by forming tight complexes with them. Since the antidotes are labile, newborn plasmid-free cells experience decay of the antidotes. This activates the toxins that, in turn, kill the plasmid-free cells (42). The proteic systems were originally identified on plasmids (6, 47, 53, 64, 80, 88), and a number of chromosomal counterparts were subsequently discovered (29, 51, 89). The function of the chromosome-encoded systems is unknown, but they appear to be dispensable for cell growth and viability under laboratory conditions (51). Recent findings indicate that plasmid-encoded restriction-modification systems mediate plasmid stabilization (46, 56). The stabilization phenotype was found to be due to killing of plasmid-free cells. The selective killing is a consequence of the dilution of the modification enzyme in growing plasmid-free cells. Below a certain level, the modification enzyme cannot protect the host cell from detrimental cleavage by the restriction enzyme.

Some ten years ago, we discovered yet another type of plasmid-encoded system that mediates efficient plasmid stabilization by killing plasmid-free cells (24, 26). In these systems, the antidotes are antisense RNAs that inhibit translation of toxin-encoding mRNAs. The antisense RNAs are unstable, whereas the toxin-encoding mRNAs are very stable. The selective killing of plasmid-free cells is based on the differential decay rates of the antisense RNAs and the mRNAs. The prototype of these latter systems is hok/sok from plasmid R1. Thirteen hok-homologous genes from

eubacterial plasmids and chromosomes have been identified to date. Interestingly, the chromosome of Escherichia coli K-12 contains five such genes. In this review, we describe the genetics, molecular biology, and evolution of the hok gene family. We emphasize the complicated RNA biology that governs the postsegregational activation of hok mRNA translation in plasmid-free cells. As will become evident, our analyses have revealed a number of interesting surprises.

THE PROTOTYPE SYSTEM: HOK/SOK FROM PLASMID R1

The hok/sok system from plasmid R1 was discovered because it can stabilize the inheritance of plasmids replicating in E. coli (24). The stabilization is very efficient and replicon independent (4, 20, 24). Low-copy-number plasmids (F and R1) are stabilized 50- to 100-fold, whereas high-copy-number plasmids (p15A and pBR322) are stabilized 1000- to 10,000-fold. Thus, hok/sok stabilizes high copy-number-plasmids with such high fidelity that they are considered technically stable (97-99). The hok/sok system mediates efficient plasmid stabilization in a wide variety of Gram-negative bacteria (20, 22). Therefore, the hok/sok system constitutes a simple and efficient tool for the technical stabilization of bacterial plasmids in a wide variety of cases, as recently reviewed in Reference 22.

The genetic organization of the hok/sok system is shown in Figure 1A. The locus codes for three genes are denoted hok, sok, and mok, respectively. The hok (host killing) gene specifies a membrane-associated toxin of 52 amino acids (aa) (26). The toxin causes irreversible damage to the cell membrane and is thus lethal to host cells (21). The sok gene (suppression of killing) codes for an antisense RNA of 64 nucleotides (nt), which is complementary to the hok mRNA leader region (23, 27). Sok-RNA is unstable, but is constitutively expressed from a relatively strong promoter. In contrast, hok mRNA is very stable and is constitutively expressed from a relatively weak promoter (23, 27). The mok (modulation of killing) reading frame overlaps extensively with hok, and it is required for expression and regulation of hok translation (49, 83). Genetic analyses showed that Sok-RNA inhibits translation of the mok reading frame and that translation of hok is coupled to translation of mok (83). Consequently, Sok-RNA regulates translation of hok indirectly via mok. The secondary structure of the 64-nt Sok-RNA is shown in Figure 1B.

PLASMID STABILIZATION BY POSTSEGREGATIONAL CELL KILLING

In 1986, we investigated the mechanism of plasmid stabilization using a pSC101 test plasmid with a temperature-sensitive replication system (26). The advantage of such a system was that synchronous plasmid-curing could be obtained simply by shifting growing cells from 30[degree]C (at which temperature the pSC101 plasmid replicated normally) to 42[degree]C (which prevented further rounds of plasmid replication, but allowed normal cell growth and division). Using this technique, it was shown that cells cured of a plasmid carrying hok/sok are rapidly killed (26). The selective cell killing prevents the proliferation of plasmid-free progeny. Phenotypically, this leads to plasmid stabilization in a growing bacterial culture. This phenomenon was coined postsegregational killing, or PSK (26). The discovery of the PSK principle behind the plasmid-stabilization phenotype was exciting: The killing appeared paradoxical, since Hok activity was triggered in cells devoid of the hok gene, and furthermore, the killing could be regarded as a terminal differentiation event initiated by plasmid loss. Other examples of programmed cell death in bacteria have been described and reviewed recently (42, 100).

PLASMID-ENCODED HOK HOMOLOGOUS GENES

Conjugative low-copy-number plasmids belonging to the IncF, IncB, and IncI incompatibility groups (plasmids that cannot coexist stably in the same cell line are said to be incompatible) carry regions that cross-hybridize with hok probes in Southern analyses (32). Over the years, six of these systems have been cloned and analyzed. The main properties of these systems are listed in Table 1 and described in some detail below.

THE FLM LOCUS OF PLASMID F

The conjugation systems of R1 (IncFII) and F (IncFI) are similar, and heteroduplex analyses have shown that the sequence homology extends into the transfer leading regions of the plasmids (19, 96). The hok/sok system is located in the transfer leading region of plasmid R1, a few kilobases (kb) downstream of the origin of transfer (oriT). Thus, during conjugation, hok/sok is transferred early. A locus from the leading region of F (designated flm for F leading maintenance) was found to stabilize the inheritance of heterologous replicons (33, 50). By comparing DNA sequences, it appeared that the flm and hok/sok loci are 95[percent] similar and that the Hok and FlmA proteins only differ by one aa (28, 50). Curiously, a variant of the flm locus (called stm for stable maintenance) has an in-frame insertion in the toxin-encoding reading frame, resulting in a killer protein with seven additional aa near the C-terminal end (33). The origin of this "in-frame" insertion in the flmA gene is not known. As in hok/sok of R1, flm and stm of F stabilize heterologous plasmids (33, 50).

THE SRNB LOCUS OF PLASMID F

In 1972, it was observed that the addition of rifampicin to certain E. coli strains resulted in membrane damage, RNase I influx from the periplasm, and degradation of stable RNA (rRNA and tRNA) (69). This syndrome is similar to the cellular changes induced by the Hok protein (21) (see discussion below). The locus responsible for the rifampicin-induced degradation of stable RNA was designated srnB (stable RNA degradation) and was found to be encoded by the sex factor F (67). Comparison of hok/sok and srnB revealed that the two loci have a similar structural organization, similar regulatory elements, and identical phenotypes (28, 59). Thus, although the systems are [similar]40[percent] dissimilar at the nucleotide level, they appear to be functionally equivalent. This is surprising, given that F also carries the flm system.

THE PND LOCI OF R483, R16, AND R64

The pnd (promotion of nucleic acid degradation) loci of R483 (IncIa) and R16 (IncB) were, like srnB of F, identified by rifampicin-induced membrane damage (66). Later structural and functional analyses showed that the pnd loci belong to the hok gene family, mediate plasmid stabilization, and encode RNAs similar to those of hok/sok (28, 59). A pnd locus very similar to pnd of R483 was recently identified on the Salmonella typhimurium virulence plasmid R64 (62).

OTHER PLASMID-ENCODED HOK-HOMOLOGOUS LOCI

A number of other plasmid-encoded hok-homologous loci have been identified. Using Southern hybridization, it was shown that [similar]50[percent] of all IncF-like plasmids are homologous in their transfer leading regions and may therefore contain hok-like genes (32). The IncI plasmid ColIb-P9 contains a locus closely related to pnd of R483 (9). E. coli cells containing the plasmids R621a (IncIg) and R483 (IncB) exhibit the typical Hok-induced damage response after the addition of rifampicin (66). Therefore, these plasmids most likely also encode hok-like loci (i.e. pnd).

Taken together, these observations show that hok-homologous gene systems are surprisingly abundant on plasmids from enteric bacteria. To date, all the plasmid-encoded systems tested mediate PSK and membrane damage upon addition of rifampicin to growing cells (see Table 1).

POSTSEGREGATIONAL CELL KILLING RELIES ON DIFFERENTIAL RNA DECAY

It was of obvious interest to elucidate the control loop that regulates postsegregational killing by hok/sok and its homologues. The sok (suppression of killing) gene encodes a trans-acting antisense RNA that inhibits hok mRNA translation (23, 26). We measured the lifetimes of the hok- and sok-encoded RNAs and found that hok mRNA is exceptionally stable (half-life in the order of hours) and that Sok-RNA is quite labile (half-life in the order of 30 seconds). These basic observations led to the proposal that induction of Hok activity in plasmid-free cells or in cells treated with rifampicin relies on the differential decay rates of the hok- and sok-encoded RNAs: in both situations, the rapid decay of the

antisense RNA results in an uninhibited pool of toxin-encoding hok mRNA freely accessible for translation. Similar differential decay rates for the antisense RNAs and mRNAs have been observed in the cases of flm, srnB, and pnd systems (59). Thus, the proposed scheme in a simple way explains the killing of plasmid-free segregants and the induction by rifampicin (23, 27). Basically, this induction model is still valid. However, the events preceding activation of hok mRNA translation in plasmid-free cells are considerably more complicated and involve a unique type of RNA metabolism, as described in the following sections.

FULL-LENGTH HOK MRNA IS TRANSLATIONALLY INACTIVE

The PSK model proposed above is based on an antidote-toxin principle by which the selective killing of plasmid-free cells is explained by the instability of the antidote. However, the interaction between Sok-RNA and hok-mRNA leads to duplex formation between the RNAs. The duplexes are rapidly cleaved by RNase III both in vitro and in vivo (25). Thus, as described for numerous antisense RNA gene systems, the proposed scheme of inhibition leads to irreversible mRNA inactivation and decay (3, 7, 45, 92). Therefore, the initial PSK model did not explain how hok translation is activated in plasmid-free cells. The answer to this important paradox came from the observation that hok mRNA exists in two forms (25, 27). One form, denoted full-length hok mRNA, is translationally inactive and binds the antisense RNA very inefficiently (18, 25, 27, 84). The secondary structure of the inert full-length hok mRNA is shown in Figure 2B and was obtained by structure probings, mutational analyses, phylogenetic comparisons, and computer predictions (17, 18, 35, 58, 85). The 3' end specifies a so-called "fold-back-inhibition" element (fbi) that pairs with the very 5' end of the mRNA. The 5' to 3' pairing locks the RNA in an inert configuration in which the Shine-Dalgarno element of mok (SDmok) is sequestered by an upstream anti-SD element denoted ucb (upstream complementary box). The Shine-Dalgarno element of hok (SDhok) is sequestered by a nearly perfect repetition of the ucb element denoted dcb (downstream complementary box). The fold-back inhibitory element also sequesters the antisense RNA recognition element in hok mRNA (sokT), thus preventing antisense RNA binding (Figure 2B). Therefore, full-length hok mRNA is inert with respect to translation and antisense RNA binding. Since the mRNA is inert, it can accumulate without killing the host cells, and it simultaneously avoids inactivation due to antisense RNA binding. As described below, the accumulation of a reservoir of an activable full-length hok mRNA is a prerequisite for the postsegregational killing mechanism.

ACTIVATION OF TRANSLATION

TRANSLATION OF HOK IS ACTIVATED BY MRNA 3' PROCESSING

A second version of the hok mRNA is generated by slow 3' processing of the full-length mRNA (27). The 3' processing leads to removal of the 40 terminal nucleotides of the full-length mRNA (see Figure 2B, C). The truncated mRNA is stable, translationally active, and binds Sok-RNA avidly (18, 25, 27, 84).

E. coli cells contain two 3' exoribonucleases involved in mRNA metabolism, polynucleotide phosphorylase (PNPase), and ribonuclease II (34). Recently, we investigated the 3' processing of hok and pnd mRNAs using E. coli host cells mutated in either pnp (encoding PNPase) or rnb (encoding ribonuclease II), or both (ND Mikkelsen & K Gerdes, unpublished). Inactivation of either one of the exonucleases did not prevent the 3' processing, whereas the simultaneous inactivation of both enzymes abolished processing. Thus, PNPase and ribonuclease II can both accomplish the 3' processing of hok and pnd mRNAs. Usually, 3' processing by PNPase and ribonuclease II leads to mRNA inactivation and decay (10, 14, 52). Therefore, the hok family of mRNAs constitutes an unusual case in which the opposite is true: Translation is activated by 3' exonucleolytic processing.

THE 3' PROCESSING TRIGGERS STRUCTURAL REARRANGEMENTS AT THE MRNA 5' END

Single point-mutations in the very 5' end of hok mRNA prevent translation of mok and therefore also translation of hok (17). This mutational

analysis defined a translational activation element (tac) at the 5' end of the mRNA (Figure 2B, C). The tac element is located [similar]100 nt upstream of the SDmok element, which constitutes its target of regulation. Besides being complementary to the 3' fbi element, tac is also partly complementary to the ucb element that sequesters SDmok in full-length hok mRNA (Figure 2B). The 3' processing, which disrupts the fbi-tac pairing, might therefore trigger refolding of the mRNA 5' end such that tac pairs with ucb in the truncated mRNA. The refolding, in turn, could mediate rearrangements further downstream in the RNA with the formation of structures that would allow translation and antisense RNA binding. This proposal was supported by folding simulations by the so-called genetic algorithm (36). Using the structure of the full-length mRNA as the input (Figure 2B), the algorithm was asked to simulate the 3' processing by progressive shortening of the length of the RNA chain by a few nucleotides at each successive calculation of intermediate structures (for details, see Reference 35). Thus, the algorithm disrupted the fbi-tac interaction by stepwise shortening of the RNA chain until the 3' end of the truncated mRNA was reached. This type of analysis was accomplished for hok mRNA as well as for many of the other plasmid- and chromosome-encoded mRNAs.

It appeared that the simulated 3' processing predicted similar structural rearrangements of the 5' untranslated regions in all cases. The refolded, truncated hok mRNA is shown in Figure 2C. The partial tac-stem already present at the 5' end of the full-length mRNA was extended, thus giving rise to the entire tacstem in which the very 5' end pairs with ucb. Thus, the refolding disrupted the ucb/SDmok pairing known to prevent translation (17, 58). The in vivo genetic analyses and the in vitro secondary structure analyses confirmed the presence of the tac-stems in the refolded hok and pnd mRNAs (18), and sequence comparisons support the hypothesis that all the hok-related mRNAs form similar tac-stems (see Figure 3).

STRUCTURE AND FUNCTION OF THE ANTISENSE RNA TARGET

The refolding of the 5' end of hok mRNA was accompanied by significant structural rearrangements in the translation initiation regions located downstream of the tac-stem. A stem-loop structure, denoted the antisense RNA target hairpin, appeared in the folding simulations (Figure 2C). Structural analyses confirmed the existence of the target hairpin (18), and sequence comparisons show that similar hairpins are present in all hok-related mRNAs (Figure 3).

In the target hairpin, SDmok is located at the bottom of the 5' part of the antisense RNA target-stem and is sequestered by the dcb element (Figure 2C). Usually, such sequestration leads to inhibition of translation (12, 13, 30). However, mutations in dcb, which disrupted the pairing, abolished translation of truncated hok mRNA (18). Structural analyses indicated that the dcb mutations changed the overall secondary structure of the truncated RNA in favor of the ucb/SDmok pairing. This indicates that the SDmok/dcb pairing in the target-stem is required to maintain truncated hok mRNA in a translatable configuration (18). This conclusion was corroborated by analyses in which the tac-stem was forced by mutation. In such engineered mRNAs, the dcb mutations activated translation dramatically (18).

All antisense RNA target-stems in the hok-homologous mRNAs are between 13 and 16 base-pairs (bp) long and topped by loops between 7 and 9 nucleotides (see Figure 3). More important, the loops are in all cases complementary to the very 5' ends of the cognate antisense RNAs. The nucleotides complementary to the very first nucleotide in the antisense RNAs are in all cases rCs located at similar positions in the loops (Figure 2C, Figure 3). The similarity of the stem-loops suggests that the cognate antisense RNAs recognize these structures in all cases.

PHYLOGENETICALLY CONSERVED FOLDING PATHWAY

METASTABLE HAIRPINS IN THE NASCENT TRANSCRIPTS

The tac-stem, which is required for activation of translation, is located at the 5' end of hok mRNA (Figure 2C). If formed in the nascent transcript, the tac-stem predictably would lead to formation of the antisense RNA target stem-loop further downstream, thereby leading to premature antisense RNA binding or to translation of hok. Obviously, both

these scenarios would be detrimental to the PSK mechanism. How, then, is premature tac-stem formation prevented?

It appears that hok mRNA can form a local 5' hairpin as an alternative to the tac-stem (Figure 2A) (35). Similar hairpins were found in all hok-homologous mRNAs, and their existence is strongly supported by nucleotide covariations that preserve base-pairing (Figure 3). Estimates of folding kinetics suggest that the 5' hairpins are long-lived and probably exist until completion of the transcripts (i.e. the hairpins are metastable). This suggests that the metastable hairpins persist until they are disrupted by the establishment of the fbi-tac interactions in the full-length transcripts (35).

Using phylogenetic comparisons and RNA folding simulations, we delineated a "consensus" folding pathway for the hok family of mRNAs (35). The main conserved intermediate structures of the folding pathway are visualized in Figure 2, using hok mRNA as an example. In the 5' untranslated region, the folding of the metastable hairpin at the very 5' end prevents tac-stem formation and favors the formation of the ucb/SDmok and dcb/SDhok interactions (Figure 2A). These hairpins are also present in the full-length mRNA (Figure 2B). Therefore, during mRNA synthesis, the antisense RNA target hairpin is not formed (Figure 2A versus 2C). Presumably, this simultaneously reduces the rate of antisense RNA binding and prevents ribosome loading to the nascent transcript (35).

FOLDING OF THE CODING REGIONS

The folding simulations predicted that the coding regions of all hok-homologous mRNAs fold into similar and very stable structures (35). Secondary structure analyses supported the presence of the predicted structure in hok mRNA (85). In all RNAs, the coding regions were closed by stable pairings between their 5' and 3' ends, thus representing separate domains of about 140 nucleotides in the secondary structures. The 5' to 3' pairing of the coding regions may facilitate the formation of the fbi-tac interactions in the full-length mRNAs (Figure 2B).

FOLDING OF THE FULL-LENGTH MRNAS

In full-length RNAs, the 5' tac-elements were predicted to pair with the 3' fbi-elements for all mRNAs. This interaction resulted in disruption of the metastable hairpins, and caused further refolding of the 5' regions with partial formation of the tac-stems. The refolded full-length hok mRNA is shown in Figure 2B. The fbi-tac pairings are strongly supported by nucleotide covariations in the RNAs (Figure 3) and were recently verified experimentally in the hok and pnd mRNAs (17, 18).

FOLDING OF THE TRUNCATED MRNAS

Folding simulations suggest that the 3' processing of full-length hok mRNA triggers a rearrangement of its 5' end with formation of the tac-stem and the antisense RNA target stem-loop (see Figure 2C) (35). The target stem-loop allows rapid antisense RNA binding or translation (in the absense of antisense RNA). Similar refolding events triggered by 3' processing were predicted for all plasmid-encoded hok-homologous mRNAs and for all chromosome-encoded mRNAs, except the E. coli hokD (relF) mRNA, whose 5' end is not homologous to those of the other mRNAs.

Direct experimental evidence was obtained for all the postulated structures in hok mRNA except in the case of the metastable structure (17, 18, 58, 85). However, the phylogenetic data presented below provide reliable support for the importance of the proposed transient foldings.

A LARGE NUMBER OF NUCLEOTIDE COVARIATIONS SUPPORT THE SECONDARY STRUCTURES

The alignment of 9 of the 13 known hok-homologous mRNAs is shown in Figure 3. Because of close similarities with other mRNAs of the family, hokA of E. coli K-12 and pnd of the Salmonella typhimurium plasmid R64 were omitted from the alignment. The hokD (relF) and hokE loci were omitted because they lack essential regulatory elements (see later). All known hok-homologous sequences are derived from E. coli plasmids or chromosomes, or from bacteria closely related with E. coli. Therefore their large

sequence diversity appears quite surprising. Furthermore, despite such diversity, the overall genetic organization of the systems has been conserved. This suggests that a strong evolutionary selection pressure ensures the overall structural conservation of the systems.

The alignment in Figure 3 clearly demonstrates the presence of the conserved, mutually exclusive secondary structures in the mRNAs. By close scrutiny of the aligned sequences, a large number of nucleotide covariations that support the postulated secondary structures were disclosed (35). Covarying bases (different bases that form base-pairs at the same position in similar stems) are shown in color in Figure 3. As seen from the Figure, all structures contain at least three covarying base-pairs. Two or more covariations in similar secondary structures of phylogenetically related RNAs are generally accepted as reliable proof for their existence (41, 60, 95). The nucleotide covariations in the metastable hairpins are particularly important, since their transient nature renders it relatively difficult to obtain direct chemical evidence for their existence.

COUPLED COVARIATIONS YIELD STRONG SUPPORT FOR THE FOLDING PATHWAY

The sequence alignment further reveals that some nucleotides covary with two partners (called coupled covariations and shown in red in Figure 3). For example, the unique C residue in pnd at the position corresponding to U141 in hok (in the dcb element) is paired to unique Gs upstream (nt 106) in the antisense target stem-loop or downstream (nt 169) in the stem that blocks the SD-element of the toxin gene. Other covariations are even more complicated. For example, the covariation of pair 5-71 in the tac-stem is coupled with covariations of pairs 71-109 in the ucb/SDmok pairing and with pair 5-392 in the fbi-tac interaction. Taken together, the coupled covariations provide evidence that the 5' tac-elements pair with three different partners during the folding path as visualized in Figure 2: (a) in the metastable structures during transcription, (b) in the long-range fbi-tac interaction in full-length RNAs, and (c) with the ucb elements in the tac-stems in truncated RNAs. Furthermore, the coupled covariations show that these mutually exclusive structures coevolve such that the overall folding pathway is preserved (15).

ANTISENSE RNA BIOLOGY

SECONDARY STRUCTURE

The secondary structure of Sok antisense RNA from plasmid R1 is shown in Figure 1B. The RNA consists of a 5' single-stranded leader of 11 nt followed by a hairpin with a stem of about 20 base-pairs with two looped-out nucleotides (86). The stem-loop is followed by several rU's, which may ensure termination of transcription. In addition, the stable stem proposedly prevents the single-stranded 5' end from interacting with other parts of the molecule, thereby ensuring that the 5' end is ready to interact with the target-loop in truncated hok mRNA. Computer analyses predict similar foldings for all the Sok-homologous antisense RNAs.

Figure 4 shows the alignment of the Sok-homologous antisense RNAs. The alignment reveals a number of covariations (shown in boldface) that support the proposed foldings of the RNAs. Thus, it can be concluded that all plasmid- and chromosome-encoded Sok-homologous antisense RNAs have similar secondary structures (Figure 1B), in accordance with previous work (59). In the antisense RNAs, the stems are the equivalents of (i.e. complementary to) the ucb/SDmok stems in the corresponding mRNAs.

METABOLISM BY RNASES AND POLY(A) **POLYMERASE** I

The Sok-homologous antisense RNAs are characterized by high turnover rates with typical half-lives of less than one minute (27, 59). The instability is due to processing by intracellular RNases. Using E. coli host cells mutated in the rne gene (encoding RNase E), it was shown that Sok-RNA is functionally inactivated by RNase E-mediated endo-cleavage at its 5' single-stranded leader (54). The position of cleavage, 6 nt from the 5' end of Sok-RNA, is indicated in Figure 1B. RNase E cleaves RNA I from ColE1 5 nts from its 5' end, which is also in a single-stranded configuration (48, 87). Furthermore, as in the case of RNA I, Sok-RNA is polyadenylated by poly(A) **polymerase** I (PcnB) in vivo (54). The

polyadenylation renders the downstream RNase E cleavage product an efficient substrate for polynucleotide phosphorylase which rapidly degrades the RNA from its 3' end. Thus, the metabolism of Sok-RNA exhibits a striking resemblance to that of RNA I of ColE1 in all major aspects investigated (10).

MECHANISM OF ANTISENSE RNA BINDING

Antisense RNAs responsible for plasmid-replication control usually recognize their target RNAs via an initial loop-loop interaction (15, 73, 92). The initial complex (the so-called kissing complex) is rapidly converted to a more stable configuration that involves duplex formation between the two RNAs. Usually, a single-stranded region near the 5' end of the antisense RNA recognizes the complementary region in the target RNA, and from there on more complete duplexes are formed. Thus this type of antisense RNA-binding mechanism involves two steps. In contrast, Sok-RNA recognizes its target RNA via its 5' single-stranded region by a one-step binding mechanism (18, 86). The structure of the antisense RNA target stem-loop in hok mRNA is shown in Figure 2C. The nucleotides in hok mRNA that are complementary to the very 5' end of Sok-RNA are presented in a loop of 7 nt (the target-loop) and are thus probably in a configuration that can be recognized by the single-stranded 5' end of Sok-RNA (18, 35). After the initial recognition reaction between the RNAs, they form a more extensive duplex. Therefore, in the killer systems, the site of initial recognition between the RNAs is coincidental with the nucleation point of duplex formation. Since the duplexes are rapidly cleaved by RNase III, antisense RNA binding confers irreversible mRNA inactivation and decay (25).

The Sok-RNA/hok mRNA-binding mechanism resembles the RNA-IN/RNA-OUT pairing that regulates transposase expression in the mobile genetic element Tn10 (7, 8, 43, 92). In the latter case, the target is the single-stranded 5' end of the tnp mRNA (RNA-IN), which is recognized by a hairpin loop in the antisense RNA, RNA-OUT. Therefore, the situation is the reverse of the Sok-RNA/hok mRNA pairing. Other features are similar: The single-stranded 5' end of one molecule interacts with a loop in the other RNA, and the first contacts are realized with stable G-C base-pairings. According to the mechanism of RNA/RNA duplex initiation and propagation, these systems define a distinct group that is different from the group of RNA pairs that recognize their partner via kissing complex formation (92).

MOLECULAR MODEL THAT EXPLAINS PROGRAMMED CELL DEATH

Based on the preceding sections, we can now delineate in molecular detail the series of events that lead to activation of hok translation in plasmid-free cells (visualized in Figure 5). First, a reservoir of the inert full-length hok mRNA accumulates in plasmid-carrying cells. This RNA is continuously processed at its 3' end by the exonucleases, thereby leading to generation of the truncated RNA. After refolding, this RNA contains the antisense RNA target hairpin and therefore is active with respect to translation and antisense RNA binding. In plasmid-carrying cells, Sok-RNA rapidly binds to hok mRNA via the target- loop and thereby prevents its translation. The immediate RNase III cleavage of the duplexed RNAs explains why truncated hok mRNA is not detectable in growing cells (25). Plasmid-free cells that arise by division of plasmid-carrying cells inherit pools of both the inert full-length hok mRNA and the Sok antisense RNA. Due to its instability, the antisense will decay rapidly. Therefore, the continued slow 3' processing of full-length hok mRNA leads to accumulation of the stable truncated mRNA. This, in turn, leads to hok translation and selective killing of the plasmid-free cells. Note that the kinetics of the 3' exonucleolytic processing event is important, since mutations (in the fbi element) that lead to rapid truncation inactivate the PSK mechanism by depleting the reservoir of activable full-length hok mRNA (17, 58).

MULTIPLE CHROMOSOME-ENCODED LOCI

A number of chromosome-encoded hok-homologous gene systems have been identified and analyzed (listed in Table 1). The chromosome of E. coli K-12 contains five such loci. Even though these loci certainly encode

hok-like killer genes, the proteins are quite distantly related (see Figure 6C). The presence of chromosome-encoded homologues of plasmid-encoded maintenance systems (also including centromere-like partition systems, proteic killer systems, and site-specific recombination systems) is the rule rather than the exception. For example, homologues of the plasmid P1 partition system are present on the chromosomes of Caulobacter crescentus, Pseudomonas aeruginosa, and Bacillus subtilis (39, 44, 54a, 63). In two cases it has been shown that these systems function to stabilize the inheritance of their chromosomes (39, 54a). Furthermore, two copies of the proteic plasmid stabilization system pem of R100 are present on the E. coli chromosome (51). However, we find the presence of five hok-like gene systems on the E. coli chromosome surprising; it may point to some hitherto uncovered function or feature of this type of gene cassette. The biology of the chromosomal hok-homologous gene systems is briefly described below.

HOKA OF E. COLI K-12 AND E. COLI C

A hok-homologous reading frame located just downstream of the cspA locus at 80.1' at the E. coli K-12 chromosome was identified by database searching (5, 72). We called the gene hokA, and its putative product of 51 aa was denoted HokA. Using **polymerase** chain reaction (PCR), the structural hokA gene was cloned into a conditional expression vector. Expression of HokA led to the typical Hok-induced cell-damage response (72). Sequence comparisons showed that an IS150 element is located a few bp upstream of the hokA reading frame. Inspection of the nucleotide sequence upstream of the IS-element revealed that the element had transposed into the middle of a gene system similar to the other hok-homologous loci. Thus, the hokA locus contains putative fbi and tac elements. However, sequence alignments also indicated the presence of a 40-bp deletion adjacent to the IS-element, which would inactivate the putative antisense RNA promoter and remove the 5' part of the putative mok-homologous reading frame. Obviously, such a deletion would inactivate the hokA locus with respect to the PSK mechanism.

A collection of E. coli strains was screened by PCR for the presence of hokA loci without an IS150 element. We found that all E. coli K-12, K-10, and B strains probably contain hokA loci with the IS150 element. However, E. coli C appeared to contain a hokA locus without the IS150 element. Further cloning and analysis of this locus revealed that it encodes an intact killer gene, a mok-homologous reading-frame, which we denote mokA, and an unstable SokA antisense RNA of 55 nt (Figure 4). Northern analyses showed that full-length hokA mRNA is processed at its 3' end to a stable truncated mRNA (72). The alignment of hokA mRNA with the other mRNAs reveals that all major regulatory elements are present, thus indicating that hokA of E. coli C (and K-12) was derived from an active PSK system (Figure 3). For reasons unknown, hokA from E. coli C was not clonable in E. coli K-12 (72).

HOKB OF E. COLI K-12

A second hok-homologous reading frame, denoted hokB, was found by data base searching, to be located near the cybB gene (encoding cytochrome b561) at 32.1' (K Gerdes, unpublished data). Alignment of the putative hokB mRNA with the other hok-homologous mRNAs revealed all the regulatory elements as described previously for these mRNAs (Figure 3). The mok-homologous reading frame was denoted mokB, and the putative antisense RNA was called SokB (Figure 4). The hokB locus has not yet been cloned and analyzed.

HOKC (GEF) OF E. COLI K-12

The gef (gene fatale) locus at 0.4' at the E. coli K-12 map was identified by Southern analysis using R1 hok-encoding DNA as a hybridization probe (74). It was shown by PCR cloning that gef-homologous loci are present in E. coli B and C. Furthermore, indirect evidence (i.e. Southern hybridization) suggested that gef might be conserved outside of the family of enteric bacteria (74). However, the specificity of these findings remains to be confirmed, and at present there is no direct evidence as to the existence of hok-like genes in Gram-negative bacteria other than the enterics. This interesting supposition awaits further analyses.

The gef locus of E. coli K-12 was cloned and analyzed (74, 75). A

mok-homologous reading frame (denoted orf69) overlaps with gef and, as in the case of hok, translation of gef was found to be coupled to that of the overlapping reading frame. Furthermore, a trans-acting antisense RNA that regulates translation of orf69 was also identified. The antisense RNA gene was called sof (suppression of fatality). Thus the function of orf69 is similar to that of mok in the hok/sok system. To create a uniform genetic nomenclature, we suggest here that gef, sof, and orf69 be renamed hokC, sokC, and mokC, respectively.

An IS186 element is located 20 nt downstream of the hokC reading frame in E. coli K-12 (74). As in the case of hokA, we subtracted the sequence of the IS186 element from the contig encoding hokC. Inspection of the hokC locus revealed the presence of all the structural elements found in hok mRNA (Figure 3). The hokC- and SokC-RNAs are aligned with the other RNAs in Figures 3 and 4, respectively.

HOKD (RELF) OF E. COLI K-12

The E. coli relB operon consists of three genes, denoted relB, relE, and relF (2). Mutations in the first gene confer the so-called "delayed relaxed response" upon the host cells (2). By visual inspection of the DNA sequence, the third gene, relF, was identified as a hok-homologous gene (21). To obtain a uniform genetic nomenclature, we suggest that relF be renamed hokD.

The hokD (relF) gene was cloned in a conditional expression vector and shown to encode a protein whose expression elicits the typical Hok-induced damage response (21). Recently, we realized that a putative fbi sequence is located downstream of the hokD gene. However, no putative tac sequence was found upstream of hokD, and the relB locus does not appear to encode an antisense RNA (59). The presence of the fbi sequence suggests that hokD of E. coli K-12 is derived from an intact hok-homologous gene system regulated by a mechanism similar to that of the other PSK systems. This finding further suggests that the present-day hokD locus probably constitutes an evolutionary relic of an ancient hok-homologue.

HOKE OF E. COLI K-12

A fifth hok-homologous reading frame, denoted hokE, was identified by data base searching of the entire E. coli K-12 genome. The hokE locus, which is located at 13.1', contains putative tac and fbi regulatory elements, but exhibits irregularities in other elements. Because the meaning of this is not yet clear, we chose not to include the hokE RNAs in the analyses presented here. Curiously, an IS186 element is located just downstream of the hokE reading frame, in a position similar to that of the IS186 element located just downstream of hokC. The HokE protein is quite distantly related to the other Hok-like proteins and is most similar to the SrnB outgroup (see Figure 6).

HOKH OF HAFNIA ALVEI

A hok-homologous reading frame, denoted hokH, was found by data base searching, located just downstream of the lysine decarboxylase gene of the enteric bacterium H. alvei (K Gerdes, unpublished data). Alignment of the putative hokH mRNA with the other mRNAs is shown in Figure 3; it contains all structural elements necessary for the folding pathway (including tac, fbi, metastable hairpin, and the ucb/SDmok target hairpin). However, no mok-homologous reading frame is present in the published sequence. Thus the hokH locus may, as in the cases of the five hok-homologous loci of E. coli K-12, constitute an inactivated PSK system. The hokH system has not yet been cloned and analyzed.

MECHANISM OF CELL KILLING

THE TOXINS

The Hok-like proteins are small membrane-associated polypeptides of [similar]50 aa (28). They consist of two domains: an N-terminal trans-membrane a-helix, and a C-terminal domain that protrudes into the periplasm (76). The a-helical domains are hydrophobic, but flanked by positively charged amino acids, thus conforming to the "positive-inside" rule of von Heijne (91). Mutations that change the charged amino acids flanking the hydrophobic domain abolish or reduce the toxicity of the HokC protein (76). The charged amino acids may be required to fix the proteins

in their trans-membrane configuration. The periplasmic domain contains both positively and negatively charged aa, and is thus highly hydrophilic. The Hok-like proteins contain an invariant sequence motif Ala-Tyr-Glu near their C-terminal ends. Mutations that result in changes in this motif suppress or reduce toxicity (76; K Gerdes, unpublished).

THE MECHANISM OF CELL KILLING

The Hok-like proteins are very toxic to most Gram-negative species (20, 22, 26, 75, 78) and also to some extent to Gram-positive bacteria (55, 78). Induction of Hok leads to loss of the cell membrane potential, arrest of respiration, efflux of small molecules (i.e. Mg2+ and ATP), and influx of small extracellular molecules (i.e. ONPG) and even influx of periplasmic proteins such as RNase I (21, 65, 66). By phase contrast microscopy, we observed that the cells change morphology to so-called "ghost-cells" after induction of Hok protein synthesis. These ghost cells are characterized by condensed cell poles and a centrally located clearing, and resemble the ghost cells (empty cell shells) formed after induction of the lysis gene of Fx174 (101). Furthermore, the srnB gene of F complements mutations in the lS gene (68), which encodes a **holin** (101). Thus the Hok family of proteins bears functional resemblance to this large group of bacteriophage-encoded proteins, collectively known as **holins** . The **holins** create holes or pores in the inner cell membrane, thereby releasing a phage-encoded endolysin to the periplasm, where it degrades the cell wall (101). Thus, all our observations suggest that the Hok-like proteins kill the cells by mediating irreversible damage to the host cell membrane.

Two models have been proposed to explain the cell-killing activity of the Hok-like proteins: The pore model presupposes that an oligomeric form of the toxins forms trans-membrane pore-like structures that permeabilize the membrane. This is similar to the proposed mode of action of the bacteriophage-encoded **holins** (excellently reviewed in References 101, 102). The target model presupposes that the toxins interact with a specific target located in the cell membrane or in the periplasm.

In an effort to identify the cellular target of the Hok-like proteins, HokC-(Gef)--resistant host cells were selected by repeated induction of HokC (77). Two different E. coli K-12 host cell lines were used, MC1000 and C600. HokC-resistant cell lines derived from C600 were unstable, meaning that the cells consistently reverted to sensitivity after a number of generations (K Gerdes, unpublished). However, after seven cycles of repeated induction, a stable HokC-resistant derivative of MC1000 was finally obtained and denoted NWL37 (77). The resistant strain tolerates elevated amounts of HokC (Gef), Hok, PndA, and SrnB' (77). However, the strain appears sensitive to high amounts of killer protein. Curiously, transduction of the resistance phenotype to strains other than MC1000 resulted in unstable inheritance of the phenotype. These results indicate that stable inheritance of the Hok-resistance phenotype requires additional host mutations, and that MC1000 by default contains these extra mutations. The mutation resulting in stable HokC resistance was mapped to a hitherto uncharacterized reading frame (orf178) located at 55.2' on the E. coli map. The resistant host cells contain a conservative change of Asp-64 to Glu in orf178 (77). The putative orf178 gene product exhibits similarity to the RibG proteins from E. coli and Bacillus subtilis, two enzymes involved in riboflavin biosynthesis (K Gerdes, unpublished observation). The orf178 gene product is not essential since its gene can be deleted without deleterious effects on the host cells. However, orf178 probably does not code for the cellular target of the Hok proteins, since strains deleted of orf178 are sensitive to HokC- (and Hok-) mediated cell killing (77).

EVOLUTION OF THE HOK GENE FAMILY

PHYLOGENETIC RELATIONSHIPS

The highly conserved structural features of the hok-related mRNAs and the toxins encoded by them suggest a common ancestral gene. However, reconstruction of their phylogeny is not straightforward. A simple search for the most parsimonious phylogenetic tree reveals several possible solutions, with similar values of tree lengths (Figure 6). Presumably, this is a consequence of so-called homoplasy (reversions, convergence, and parallel changes) in the sequences that are under two selective pressures:

structural requirements for mRNA folding and coding of functional protein. Therefore the tree based on the alignment of the noncoding regions (Figure 6A) is different from those based on the nucleotide sequences of the coding parts or by the proteins themselves (Figure 6B, C, respectively). A comparison of the tree lengths, calculated for the same regions of RNA sequences, does not give strong preference to any of the trees. In the noncoding region, the most parsimonious tree (Figure 6A) contains 347 nucleotide substitutions, whereas both the other trees have 379 changes. In the coding part, the situation is reversed. The trees shown in Figure 6A, B, and C have 374, 340, and 346 substitutions, respectively [note that hokD (relF) was also included]. Thus being applied for the entire mRNAs, all three trees have comparable lengths. It remains to be determined which of the three is the correct one. However, this determination requires more complicated approaches that take into account assumptions on homoplasy and nonequal substitution rates in RNA structures (37, 90).

Even though the precise phylogenetic relationships in the hok gene family are not clear, some conclusions can be drawn. All three trees separate srnB as an outgroup, whereas none of them distinguishes chromosomal genes in a separate group distinct from the plasmid-encoded systems. Similarly, hokH from H. alvei does not form an outgroup. It is noteworthy that the two systems from F (flm and srnB) are quite diverse (Figure 6). All known pnd genes are closely related, and two of the chromosomal genes from E. coli K-12, hokC (gef), and hokD (relF), form a subgroup.

FUNCTION OF THE PLASMID-ENCODED PSK SYSTEMS

Compared to the apparent diversity at the level of primary structure, the strong conservation of the structural elements is very surprising. In the plasmid-encoded gene systems, the conservation is a prerequisite for the functioning of the PSK mechanism. An interesting question arises why such "selfish" behavior of plasmids is so strongly conserved, and whether these genes are really "selfish." The problem is even more intriguing because other bacterial systems that mediate programmed cell death share the main properties with the antisense RNA-regulated gene systems. In the proteic plasmid stabilization systems, the mechanism also involves a stable toxin but a labile protein antidote (reviewed in Reference 42). Recent reports demonstrate that well-known restriction modification systems can mediate plasmid stabilization by PSK. In the latter case, the antidote is a DNA methylase that prevents the lethal DNA digestion by the corresponding restriction enzyme. The PSK phenomenon is triggered by dilution of the methylase in plasmid-free cells, which allows the restriction enzyme to degrade the cellular DNA (46, 56). Such diverse ways of plasmid "selfishness," which makes the bacterial cells addicted to plasmid presence, suggest that the plasmid-bacterium pair may be considered as a symbiotic parasite-host couple with mutual benefits (for discussion, see Reference 100). For the plasmid, the main benefit is obvious: It "enslaves" the host to reproduce it. For the host, the advantage of bearing a plasmid could be an increased evolutionary potential. Also, stabilization of the plasmid may be necessary if the plasmid is advantageous in some stress conditions only, but normally is a burden for the cell.

FUNCTION OF THE CHROMOSOME-ENCODED PSK SYSTEMS

The presence of numerous hok-homologues on the bacterial chromosome suggests that these genes themselves could be beneficial. The list in Table 1 shows that all plasmid-encoded systems are active with respect to PSK, and that none of the chromosome-encoded systems has this property. It is difficult to imagine that bacterial chromosomes with their compact genetic information carry numerous gene systems without present or past function. Despite considerable effort, we have not yet identified hok-homologous systems that are active with respect to PSK from a chromosome. We thus favor the idea that the chromosomes recruited the PSK systems from conjugative low-copy-number plasmids, and that the systems were inactivated when resident on their present-day chromosome. In our view, this leaves open the possibility that active chromosome-encoded PSK systems may, under certain conditions, specify a beneficial function. If this function is connected with cell killing, then their rapid inactivation would be selected for and thus would explain the lack of PSK activity. One

possibility is that PSK systems could provoke altruistic suicide of the major fraction of a bacterial population. Survivors from such events would have an increased probability of carrying an inactivated PSK system. Recently, evidence was obtained that the hok/sok system mediates exclusion of bacteriophage T4 (71). The exclusion phenotype may be related to the PSK mechanism in the following way: Infection by the phage leads to a rapid cessation of host cell transcription and a switch to phage mRNA synthesis. This, in turn, leads to depletion of the Sok-RNA pool and subsequent activation of the PSK mechanism. If killing occurs before mature phages have developed then such altruistic suicide would prevent or reduce the spread of the virus in the bacterial population. It is not known if the PSK systems elicit such a response in natural populations, but the exclusion was not very efficient, even when the hok/sok system was present on a high-copy-number plasmid (71). However, these observations do not explain the fact that all known plasmid-encoded systems are active and that all chromosome-encoded systems are inactive with respect to PSK. Thus, the function of the chromosome-encoded systems remains an enigma that has to be addressed experimentally.

HOK-LIKE PROTEINS IN MITOCHONDRIA

In searching for a mechanism that could secure stable inheritance of mitochondrial genomes, it was observed serendipitously that the A8 subunits of mitochondrial ATP synthases exhibit weak similarity to the Hok-like proteins (40). It was argued that these essential components of the ATP synthases may have evolved from an ancestral PSK system into the present-day proteins. Although the hydropathy profiles of the Hok-like proteins and the A8 subunits are strikingly similar, the homologies at the level of primary sequences are virtually insignificant (40). Therefore the similarity between these two groups of membrane proteins could also have arisen by convergent evolution.

A KILLER GENE SYSTEM IN GRAM-POSITIVE BACTERIA

The conjugative low-copy-number plasmid pAD1 from Enterococcus faecalis is stably maintained because of a region termed par (94). The par locus encodes two small RNAs, RNA I and II, of 210 and 65 nt, respectively. The RNAs are convergently transcribed, and the major part of RNA II is complementary to the 3' end of RNA I (93). Expression of RNA I without RNA II is lethal to the host cells. Therefore, RNA II seems to be an antisense RNA that negatively regulates the function of RNA I. These and other observations prompted Weaver et al to suggest that par specifies a PSK system in which RNA I encodes a toxic function and that the toxicity is counteracted by RNA II. If this model holds, then the par system of pAD1 is the first example of a PSK system from Gram-positive bacteria.

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Added material

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Table 1 Plasmid- and chromosome-encoded hok-homologous loci with known DNA sequence

FOOTNOTE

a nd: not determined;

b tac denotes the translational activator elements at the mRNA 5'

ends, and fbi denotes the fold-back inhibitory sequences at the mRNA 5' ends, see the text;

c A putative fbi element is located downstream of hokD (relF).

Figure 1 A: Structural organization and regulatory elements of the hok/sok system of plasmid R1. Genetic nomenclature: mok, modulation of killing; hok, host killing gene; sok, suppression of killing; sokT, Sok-RNA target region; fbi, fold-back-inhibition element; tac, translational activation element. B: Secondary structure of Sok antisense RNA from plasmid R1. The RNase E cleavage site at the RNA 5' end is indicated. Non-Watson-Crick G-U base-pairings are shown with dots.

Figure 2 Folding pathway of hok mRNA. A: early steps in the folding of the nascent transcript showing the sequestration of the mok and hok Shine-Dalgarno regions. An M indicates the 5' metastable structure; ucb, upstream complementary box; dcb, downstream complementary box. B: Secondary structure of full-length hok mRNA showing the fbi-tac interaction, the ucb/SDmok and dcb/SDhok interactions, and the upper part of the tac-stem. C: Secondary structure of the truncated, refolded hok mRNA. The ucb/SDmok interaction is disrupted by the formation of the stable tac-stem. In turn, this leads to the formation of the antisense RNA target stem-loop containing the SDmok/dcb interaction. Arrow denoted sokT indicates the nucleotide complementary to the very first nucleotide of the 5' end of Sok-RNA and thus marks the 3' border of the Sok target region (sokT) in hok mRNA. The folding pathway of hok mRNA was from (18) and (35).

Figure 3 Alignment of plasmid- and chromosome-encoded hok-homologous mRNAs. Covarying nucleotides are shown in color. Color codes: red: all coupled covariations (bases that covary with more than one partner, see the text); green: covariations in tac and target stems; blue: covariations in ucb/SDmok and dcb/SDhok stems. Secondary structure elements in hok mRNA are indicated by colored lines above the sequences. The short arrow pointing leftward marks the nucleotides complementary to the very 5' nucleotide of the antisense RNAs. This base position in the alignment is an invariant rC. The AUG start-codon of hok, SDmok, and SDhok are indicated with dark blue lines above the sequences. Deletions are shown by dashes, and the coding parts downstream of the AUG start codons of the killer genes are symbolized by dots. Invariant nucleotides are indicated by asterisks below the sequences.

Figure 4 Alignment of the antisense RNAs encoded by the hok-homologous gene systems. Nucleotide covariations are shown in bold. The single-stranded 5' ends and the loop regions are underlined twice, and invariant nucleotides are indicated by asterisks below the sequences. The bases complementary to the mok and mok-homologous start-codons are italicized. Dashes were introduced to optimize the alignment. SokA was from E. Coli C, whereas SokB and SokC (formerly Sof) were from E. Coli K-12.

Figure 5 Model to explain induction of hok translation in plasmid-free cells. During steady-state growth, full-length hok mRNA accumulates. Because of its folded structure, hok mRNA does not bind the antisense RNA (the primary Sok-RNA target, sokT, is shielded due to the fold-back structure), and the mRNA is not translated due to the ucb/SDmok interaction. The full-length hok mRNA is processed at its 3' end in both plasmid-carrying and plasmid-free cells. The processing triggers a refolding of the mRNA into a configuration that allows translation and antisense RNA binding. A: In plasmid-carrying cells, which contain Sok-RNA, the truncated mRNA is rapidly bound by the antisense RNA. This leads to RNA duplex formation and RNase III cleavage. B: In plasmid-free cells, in which Sok-RNA has decayed, the truncated refolded mRNA accumulates. This leads to translation and killing of the plasmid-free cells ensues. Genetic symbols as in Figure 1.

Figure 6 Three alternative evolutionary trees of the hok gene family based on parsimony analysis for different regions of the mRNAs. A: Tree based on the noncoding RNA sequences; B: tree based on the coding RNA sequences; C: tree based on the protein sequences.

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--metabolism--ME; Chlorides--pharmacology--PD; DNA Primers--genetics--GE; Dipeptidyl Peptidases--genetics--GE; Escherichia coli--genetics--GE; Food Microbiology; Food Technology; Gene Expression--drug effects--DE; Gene Fusion; Genetic Markers; Lactococcus lactis--drug effects--DE; Lactococcus lactis--metabolism--ME; Osmotic Fragility; **Polymerase** Chain Reaction; Promoter Regions (Genetics)

CAS Registry No.: 0 (Chlorides); 0 (DNA Primers); 0 (Genetic Markers)

Enzyme No.: EC 3.4.14.- (Dipeptidyl Peptidases); EC 3.4.14.- (PepX dipeptidyl aminopeptidase)

Record Date Created: 19980213

induction, corresponding to an approximately 100-fold increase over the normal lethal level of **holin** . Characterization of this expression system is presented and discussed with respect to the current model of **holin** function.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Bacteriophage lambda--pathogenicity--PY; *Membrane Proteins --biosynthesis--BI; *Viral Proteins--biosynthesis--BI; Amino Acid Sequence; Bacteriophage lambda--genetics--GE; Bacteriophage lambda--metabolism--ME; Base Sequence; Cell Membrane Permeability; DNA, Viral--metabolism--ME; Electrophoresis, Polyacrylamide Gel; Endopeptidases--metabolism--ME; Escherichia coli; Gene Expression; Membrane Proteins--genetics--GE; Molecular Sequence Data; Viral Proteins--genetics--GE

CAS Registry No.: 0 (DNA, Viral); 0 (Membrane Proteins); 0 (S holin, bacteriophage lambda); 0 (Viral Proteins); 0 (bacteriophage lambda lysis effector protein S105); 0 (bacteriophage lambda lysis inhibitor protein S107)

Enzyme No.: EC 3.4.- (Endopeptidases); EC 3.4.99.- (endolysin)

Record Date Created: 19980630

32/9/19 (Item 19 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

10337725 99328986 PMID: 10400598

Lysis and lysis inhibition in bacteriophage T4: rV mutations reside in the holin **t gene.**

Dressman H K; Drake J W

Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA.

Journal of bacteriology (UNITED STATES) Jul 1999, 181 (14) p4391-6, ISSN 0021-9193 Journal Code: 2985120R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Upon infecting populations of susceptible host cells, T-even bacteriophages maximize their yield by switching from lysis at about 25 to 35 min at 37 degrees C after infection by a single phage particle to long-delayed lysis (lysis inhibition) under conditions of sequential infection occurring when free phages outnumber host cells. The timing of lysis depends upon gene t and upon one or more rapid-lysis (r) genes whose inactivation prevents lysis inhibition. t encodes a **holin** that mediates the movement of the T4 endolysin though the inner cell membrane to its target, the cell wall. The rI protein has been proposed to sense superinfection. Of the five reasonably well characterized r genes, only two, rI and rV, are clearly obligatory for lysis inhibition. We show here that rV mutations are alleles of t that probably render the t protein unable to respond to the lysis inhibition signal. The tr alleles cluster in the 5' third of t and produce a strong r phenotype, whereas conditional-lethal t alleles produce the classical t phenotype (inability to lyse) and other t alleles produce additional, still poorly understood phenotypes. tr mutations are dominant to t+, a result that suggests specific ways to probe T4 **holin** function.

Descriptors: *Bacteriophage T4--genetics--GE; *Escherichia coli--virology --VI; *Gene Expression Regulation, Viral; *Genes, Viral; *Lysogeny --genetics--GE; *Viral Proteins--genetics--GE; Amino Acid Sequence; Bacteriophage T4--physiology--PH; DNA, Viral--analysis--AN; Molecular Sequence Data; **Polymerase** Chain Reaction--methods--MT; Sequence Analysis, DNA; Viral Proteins--chemistry--CH

CAS Registry No.: 0 (DNA, Viral); 0 (Viral Proteins); 0 (t holin, bacteriophage T4)

Record Date Created: 19990802

32/9/21 (Item 21 from file: 98)

DIALOG(R)File 98:General Sci Abs/Full-Text

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DESCRIPTORS:
Plasmids; Antisense genetics; Molecular evolution; Apoptosis

32/9/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10343749 99350405 PMID: 10419939
Evidence for a holin **-like protein gene fully embedded out of frame in the endolysin gene of Staphylococcus aureus bacteriophage 187.**
Loessner M J; Gaeng S; Scherer S
Institut fur Mikrobiologie, Forschungszentrum fur Milch und Lebensmittel Weihenstephan, Technische Universitat Munchen, D-85350 Freising, Germany.
Journal of bacteriology (UNITED STATES) Aug 1999, 181 (15) p4452-60, ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
We have cloned, sequenced, and characterized the genes encoding the lytic system of the unique Staphylococcus aureus phage 187. The endolysin gene ply187 encodes a large cell wall-lytic enzyme (71.6 kDa). The catalytic site, responsible for the hydrolysis of staphylococcal peptidoglycan, was mapped to the N-terminal domain of the protein by the expression of defined ply187 domains. This enzymatically active N terminus showed convincing amino acid sequence homology to an N-acetylmuramoyl-L-alanine amidase, whereas the C-terminal part, whose function is unknown, revealed striking relatedness to major staphylococcal autolysins. An additional reading frame was identified entirely embedded out of frame (+1) within the 5' region of ply187 and was shown to encode a small, hydrophobic protein of **holin** -like function. The hol187 gene features a dual-start motif, possibly enabling the synthesis of two products of different lengths (57 and 55 amino acids, respectively). Overproduction of Hol187 in Escherichia coli resulted in growth retardation, leakiness of the cytoplasmic membrane, and loss of de novo ATP synthesis. Compared to other **holins** identified to date, Hol187 completely lacks the highly charged C terminus. The secondary structure of the polypeptide is predicted to consist of two small, antiparallel, hydrophobic, transmembrane helices. These are supposed to be essential for integration into the membrane, since site-specific introduction of negatively charged amino acids into the first transmembrane domain (V7D G8D) completely abolished the function of the Hol187 polypeptide. With antibodies raised against a synthetic 18-mer peptide representing a central

part of the protein, it was possible to detect Hol187 in the cytoplasmic membrane of phage-infected S. aureus cells. An important indication that the protein actually functions as a **holin** in vivo was that the gene (but not the V7D G8D mutation) was able to complement a phage lambda Sam mutation in a nonsuppressing E. coli HB101 background. Plaque formation by lambdagt11::hol187 indicated that both phage genes have analogous functions. The data presented here indicate that a putative **holin** is encoded on a different reading frame within the enzymatically active domain of ply187 and that the **holin** is synthesized during the late stage of phage infection and found in the cytoplasmic membrane, where it causes membrane lesions which are thought to enable access of Ply187 to the peptidoglycan of phage-infected Staphylococcus cells.

Descriptors: *Endopeptidases--genetics--GE; *Membrane Proteins--genetics --GE; *Staphylococcus Phages--genetics--GE; *Staphylococcus aureus --virology--VI; Amino Acid Sequence; Base Sequence; Cloning, Molecular; DNA, Viral--genetics--GE; Escherichia coli; Kinetics; Membrane Proteins --chemistry--CH; Membrane Proteins--metabolism--ME; Molecular Sequence Data; **Polymerase** Chain Reaction; Protein Conformation; Recombinant Fusion Proteins--biosynthesis--BI; Recombinant Fusion Proteins--chemistry--CH; Recombinant Fusion Proteins--metabolism--ME; Sequence Alignment; Sequence Homology, Amino Acid; Staphylococcus Phages--enzymology--EN; Staphylococcus Phages--physiology--PH; beta-Galactosidase--genetics--GE

Molecular Sequence Databank No.: GENBANK/Y07740

CAS Registry No.: 0 (DNA, Viral); 0 (Hol187 protein); 0 (Membrane Proteins); 0 (Recombinant Fusion Proteins)

Enzyme No.: EC 3.2.1.23 (beta-Galactosidase); EC 3.4.- (Endopeptidases); EC 3.4.99.- (endolysin)

Record Date Created: 19990819

32/9/40 (Item 40 from file: 156)

DIALOG(R)File 156:ToxFile

01230624 98069482 PMID: 9406408

A chloride-inducible gene expression cassette and its use in induced lysis of Lactococcus lactis.

Sanders J W; Venema G; Kok J

Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Haren, The Netherlands.

Applied and environmental microbiology (UNITED STATES) Dec 1997, 63 (12) p4877-82, ISSN 0099-2240 Journal Code: 7605801

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: Toxbib ; INDEX MEDICUS

A chloride-inducible promoter previously isolated from the chromosome of Lactococcus lactis (J. W. Sanders, G. Venema, J. Kok, and K. Leenhouts, Mol. Gen. Genet., in press) was exploited for the inducible expression of homologous and heterologous genes. An expression cassette consisting of the positive-regulator gene gadR, the chloride-inducible promoter Pgad, and the translation initiation signals of gadC was amplified by PCR. The cassette was cloned upstream of Escherichia coli lacZ, the **holin** -lysin cassette (lytPR) of the lactococcal bacteriophage r1t, and the autolysin gene of L. lactis, acmA. Basal activity of Pgad resulted in a low level of expression of all three proteins. Growth in the presence of 0.5 M NaCl of a strain containing the gadC::lacZ fusion resulted in a 1,500-fold increase of beta-galactosidase activity. The background activity levels of LytPR and AcmA had no deleterious effects on cell growth, but induction of lysin expression by addition of 0.5 M NaCl resulted in inhibition of growth. Lysis was monitored by following the release of the cytoplasmic marker enzyme PepX. Released PepX activity was maximal at 1 day after induction of lytPR expression with 0.1 M NaCl. Induction of acmA expression resulted in slower release of PepX from the cells. The presence of the inducing agent NaCl resulted in the stabilization of osmotically fragile cells.

Tags: Support, Non-U.S. Gov't

Descriptors: *Gene Expression; *Genes, Bacterial; *Lactococcus lactis --genetics--GE; Bacteriophages--genetics--GE; Base Sequence; Cell Wall

9667152 98069482 PMID: 9406408

A chloride-inducible gene expression cassette and its use in induced lysis of Lactococcus lactis.

Sanders J W; Venema G; Kok J

Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Haren, The Netherlands.

Applied and environmental microbiology (UNITED STATES) Dec 1997, 63 (12) p4877-82, ISSN 0099-2240 Journal Code: 7605801

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A chloride-inducible promoter previously isolated from the chromosome of Lactococcus lactis (J. W. Sanders, G. Venema, J. Kok, and K. Leenhouts, Mol. Gen. Genet., in press) was exploited for the inducible expression of homologous and heterologous genes. An expression cassette consisting of the positive-regulator gene gadR, the chloride-inducible promoter Pgad, and the translation initiation signals of gadC was amplified by PCR. The cassette was cloned upstream of Escherichia coli lacZ, the **holin** -lysin cassette (lytPR) of the lactococcal bacteriophage r1t, and the autolysin gene of L. lactis, acmA. Basal activity of Pgad resulted in a low level of expression of all three proteins. Growth in the presence of 0.5 M NaCl of a strain containing the gadC::lacZ fusion resulted in a 1,500-fold increase of beta-galactosidase activity. The background activity levels of LytPR and AcmA had no deleterious effects on cell growth, but induction of lysin expression by addition of 0.5 M NaCl resulted in inhibition of growth. Lysis was monitored by following the release of the cytoplasmic marker enzyme PepX. Released PepX activity was maximal at 1 day after induction of lytPR expression with 0.1 M NaCl. Induction of acmA expression resulted in slower release of PepX from the cells. The presence of the inducing agent NaCl resulted in the stabilization of osmotically fragile cells.

Tags: Support, Non-U.S. Gov't

Descriptors: *Gene Expression; *Genes, Bacterial; *Lactococcus lactis --genetics--GE; Bacteriophages--genetics--GE; Base Sequence; Cell Wall --metabolism--ME; Chlorides--pharmacology--PD; DNA Primers--genetics--GE; Dipeptidyl Peptidases--genetics--GE; Escherichia coli--genetics--GE; Food Microbiology; Food Technology; Gene Expression--drug effects--DE; Gene Fusion; Genetic Markers; Lactococcus lactis--drug effects--DE; Lactococcus lactis--metabolism--ME; Osmotic Fragility; **Polymerase** Chain Reaction; Promoter Regions (Genetics)

CAS Registry No.: 0 (Chlorides); 0 (DNA Primers); 0 (Genetic Markers)

Enzyme No.: EC 3.4.14.- (Dipeptidyl Peptidases); EC 3.4.14.- (PepX dipeptidyl aminopeptidase)

Record Date Created: 19980213

33/9/9 (Item 3 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04575992 Genuine Article#: TU511 Number of References: 58

Title: GENETIC-EVIDENCE FOR AN ACTIVATOR REQUIRED FOR INDUCTION OF COLICIN-LIKE BACTERIOCIN 28B PRODUCTION IN SERRATIA-MARCESCENS BY DNA-DAMAGING AGENTS

Author(s): FERRER S; VIEJO MB; GUASCH JF; ENFEDAQUE J; REGUE M

Corporate Source: UNIV BARCELONA,FAC PHARM,DIV HLTH SCI,DEPT MICROBIOL & PARASITOL/E-08028 BARCELONA//SPAIN/; UNIV BARCELONA,FAC PHARM,DIV HLTH SCI,DEPT MICROBIOL & PARASITOL/E-08028 BARCELONA//SPAIN/

Journal: JOURNAL OF BACTERIOLOGY, 1996, V178, N4 (FEB), P951-960

ISSN: 0021-9193

Language: ENGLISH Document Type: ARTICLE

Geographic Location: SPAIN

Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences

Journal Subject Category: MICROBIOLOGY

Abstract: Bacteriocin 28b production is induced by mitomycin in wild-type Serratia marcescens 2170 but not in Escherichia coli harboring the bacteriocin 28b structural gene (bss). Studies with a bss-lacZ

transcriptional fusion showed that mitomycin increased the level of bss gene transcription in S. marcescens but not in the E. coli background, A S. marcescens Tn5 insertion mutant was obtained (S. marcescens 2170 reg::Tn5) whose bacteriocin 28b production and bss gene transcription were not increased by mitomycin treatment, Cloning and DNA sequencing of the mutated region showed that the Tn5 insertion was flanked by an SOS box sequence and three genes that are probably cotranscribed (regA, regB, and regC), These three genes had homology to phage **holins** , phage lysozymes, and the Ogr transcriptional activator of P2 and related bacteriophages, respectively, Recombinant plasmid containing this wild-type DNA region complemented the reg::TnS regulatory mutant, A transcriptional fusion between a 157-bp DNA fragment, containing the apparent SOS box upstream of the regA gene, and the caf gene showed increased chloramphenicol acetyltransferase activity upon mitomycin treatment, Upstream of the bss gene, a sequence similar to the consensus sequence proposed to bind Ogr protein was found, but no sequence similar to an SOS box was detected. Our results suggest that transcriptional induction of bacteriocin 28b upon mitomycin treatment is mediated by the regC gene whose own transcription would be LexA dependent.

Identifiers--KeyWords Plus: ESCHERICHIA-COLI; NUCLEOTIDE-SEQUENCE; SATELLITE BACTERIOPHAGE-P4; EXTRACELLULAR PROTEINS; LATE PROMOTER; EXPRESSION; CLONING; REGION; RECA; CLOACIN-DF13

Research Fronts: 94-4806 002 (GENE ORGANIZATION; LONG-CHAIN FATTY-ACID TRANSPORT; TRANSCRIPTION FACTOR)
94-0736 001 (MURINE CYTOMEGALOVIRUS GENE; HUMAN PROTEIN SEQUENCES; IDENTIFICATION OF ROFA)
94-1703 001 (DNA DAMAGE-INDUCIBLE REPLICATION OF THE ESCHERICHIA-COLI CHROMOSOME; RECA GENE; UV INDUCTION)
94-6345 001 (ESCHERICHIA-COLI RNA- **POLYMERASE** ; LACUV5 PROMOTER; TRANSCRIPTION INITIATION; EXPRESSION ANALYSIS)

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33/9/10 (Item 4 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

03428067 Genuine Article#: PE570 Number of References: 64
Title: CONTROLLED EXPRESSION AND STRUCTURAL ORGANIZATION OF A LACTOCOCCUS-LACTIS BACTERIOPHAGE LYSIN ENCODED BY 2 OVERLAPPING GENES
Author(s): SHEARMAN CA; JURY KL; GASSON MJ
Corporate Source: AFRC,INST FOOD RES,NORWICH RES PK/NORWICH NR4 7UA/NORFOLK/ENGLAND/
Journal: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 1994, V60, N9 (SEP), P 3063-3073
ISSN: 0099-2240
Language: ENGLISH Document Type: ARTICLE
Geographic Location: ENGLAND
Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences; CC AGRI--Current Contents, Agriculture, Biology & Environmental Sciences
Journal Subject Category: BIOTECHNOLOGY & APPLIED MICROBIOLOGY
Abstract: The phi vML3 bacteriophage lysin is specific for lactococci and could be used to promote enzyme release during cheese manufacture. The level of lysin expression from the cloned gene using its own upstream sequences is very low. Expression in Escherichia coli by using a synthetic hybrid lysin gene and a series of BAL 31 deletions of the original cloned DNA fragment suggested that the start of the gene had previously been incorrectly assigned. Reevaluation of homology between the lysin and Bacillus subtilis PZA protein 15 led to the identification of a new potential ribosome binding site (RBS). A 0.72-kb PCR-generated fragment including this RBS and the complete lysin gene was expressed and inducibly controlled. The translational start of the lysin gene was identified as an isoleucine codon, and this may lead to a low translation rate. During the analysis of the BAL 31 deletion fragments, two proteins of 20 and 8 kDa were shown to be expressed from the originally defined lysin gene. The DNA sequence has a second open reading frame with a good RBS and two potential start methionines. The smaller lysin protein was isolated, and the N terminus was sequenced, confirming that one methionine codon acted as the start of a second gene. The larger lysin protein has homology with lysozymes. The smaller lysin protein has some features resembling those of a **holin** . The possible roles of these two proteins in lysis of lactococci are discussed.
Identifiers--KeyWords Plus: DUAL-START MOTIF; LAMBDA-S GENE; ESCHERICHIA-COLI; PHAGE PHI-29; NUCLEOTIDE-SEQUENCES; HOMOLOGOUS SEQUENCE; SECONDARY-STRUCTURE; PLASMID VECTORS; CHEDDAR CHEESE; RNA-**POLYMERASE**
Research Fronts: 92-0869 002 (LACTOCOCCUS-LACTIS SUBSP LACTIS; BACTERIOPHAGE RESISTANCE; BACTERIOCIN PLASMID)

92-3056 002 (UPTAKE OF SURFACTANT PROTEIN-B; CASEIN KINASE-II; CATALYTIC SUBUNITS)
92-3995 002 (PROTEIN SECONDARY STRUCTURE; FUNCTIONAL TOPOGENIC DOMAINS; ALPHA-HELIX PREDICTION)
92-4812 002 (PUTATIVE ANAEROBIC COPROPORPHYRINOGEN-III OXIDASE IN RHODOBACTER-SPHAEROIDES; TRANSCRIPTIONAL REGULATORY ELEMENT; FUNCTIONAL EXPRESSION)
92-8077 001 (EXPRESSION OF A RECOMBINANT GENE; VIRAL ASSEMBLY PROTEIN; VACCINIA VIRUS VECTORS; DNA-BINDING INVITRO; XENOPUS OOCYTES; DIFFERENT EXTRACELLULAR DOMAINS)

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09750839 98175413 PMID: 9515662

Bacteriophages **show promise as antimicrobial agents.**

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Document type: Journal Article; Review; Review Literature

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The emergence of antibiotic-resistant bacteria has prompted interest in alternatives to conventional drugs. One possible option is to use bacteriophages (phage) as antimicrobial agents. We have conducted a literature review of all Medline citations from 1966-1996 that dealt with the therapeutic use of phage. There were 27 papers from Poland, the Soviet Union, Britain and the U.S.A. The Polish and Soviets administered phage orally, topically or systemically to treat a wide variety of antibiotic-resistant pathogens in both adults and children. Infections included suppurative wound infections, gastroenteritis, sepsis, osteomyelitis, dermatitis, empyemas and pneumonia; pathogens included Staphylococcus, Streptococcus, Klebsiella, Escherichia, Proteus, Pseudomonas, Shigella and Salmonella spp. Overall, the Polish and Soviets reported success rates of 80-95% for phage therapy, with rare, reversible gastrointestinal or allergic side effects. However, efficacy of phage was determined almost exclusively by qualitative clinical assessment of patients, and details of dosages and clinical criteria were very sketchy. There were also six British reports describing controlled trials of phage in animal models (mice, guinea pigs and livestock), measuring survival rates and other objective criteria. All of the British studies raised phage against specific pathogens then used to create experimental infections. Demonstrable efficacy against Escherichia, Acinetobacter, Pseudomonas and Staphylococcus spp. was noted in these model systems. Two U.S. papers dealt with improving the bioavailability of phage. Phage is sequestered in the spleen and removed from circulation. This can be overcome by serial passage of phage through mice to isolate mutants that resist sequestration. In conclusion, bacteriophages may show promise for treating antibiotic resistant pathogens. To facilitate further progress, directions for future research are discussed and a directory of authors from the reviewed papers is provided. (62 Refs.)

Tags: Animal; Human

Descriptors: *Bacterial Infections--therapy--TH; *Bacteriophages --physiology--PH; Bacteriolysis; Drug Resistance, Microbial; Mice

Record Date Created: 19980428

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NiceProt View of SWISS-PROT: P50029

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[General] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

General information about the entry

Entry name	**DP3B_STAAM**
Primary accession number	**P50029**
Secondary accession numbers	None
Entered in SWISS-PROT in	Release 34, October 1996
Sequence was last modified in	Release 34, October 1996
Annotations were last modified in	Release 41, June 2002
Name and origin of the protein	
Protein name	**DNA polymerase III, beta chain**
Synonym	**EC 2.7.7.7**
Gene name	**DNAN** or **SAV0002** or **SA0002** or **MW0002**
From	Staphylococcus aureus (strain Mu50 / ATCC 700699) [TaxID: 158878] Staphylococcus aureus (strain N315) [TaxID: 158879] Staphylococcus aureus (strain MW2) [TaxID: 196620] Staphylococcus aureus [TaxID: 1280]
Taxonomy	Bacteria; Firmicutes; Bacillales; Staphylococcus.
References	

[1]	SEQUENCE FROM NUCLEIC ACID. **STRAIN**=Mu50 / ATCC 700699, and N315; MEDLINE=21311952; PubMed=11418146; [NCBI, ExPASy, EBI, Israel, Japan] Kuroda M., Ohta T., Uchiyama I., Baba T., Yuzawa H., Kobayashi I., Cui L., Oguchi A., Aoki K.-I., Nagai Y., Lian J.-Q., Ito T., Kanamori M., Matsumaru H., Maruyama A., Murakami H., Hosoyama A., Mizutani-Ui Y., Takahashi N.K., Sawano T., Inoue R.-I., Kaito C., Sekimizu K., Hirakawa H., Kuhara S., Goto S., Yabuzaki J., Kanehisa M., Yamashita A., Oshima K., Furuya K., Yoshino C., Shiba T., Hattori M., Ogasawara N., Hayashi H., Hiramatsu K.; "Whole genome sequencing of meticillin-resistant Staphylococcus aureus."; Lancet 357:1225-1240(2001).
[2]	SEQUENCE FROM NUCLEIC ACID. **STRAIN**=MW2; MEDLINE=22040717; PubMed=12044378; [NCBI, ExPASy, EBI, Israel, Japan] Baba T., Takeuchi F., Kuroda M., Yuzawa H., Aoki K.-I., Oguchi A., Nagai Y., Iwama N., Asano K., Naimi T., Kuroda H., Cui L., Yamamoto K., Hiramatsu K.; "Genome and virulence determinants of high virulence community-acquired MRSA."; Lancet 359:1819-1827(2002).
[3]	SEQUENCE FROM NUCLEIC ACID. **STRAIN**=YB886; MEDLINE=95206242; PubMed=7898435; [NCBI, ExPASy, EBI, Israel, Japan] Alonso J.C., Fisher L.M.; "Nucleotide sequence of the recF gene cluster from Staphylococcus aureus and complementation analysis in Bacillus subtilis recF mutants."; Mol. Gen. Genet. 246:680-686(1995).

Comments

FUNCTION: DNA POLYMERASE III IS A COMPLEX, MULTICHAIN ENZYME RESPONSIBLE FOR MOST OF THE REPLICATIVE SYNTHESIS IN BACTERIA. THIS DNA POLYMERASE ALSO EXHIBITS 3' TO 5' EXONUCLEASE ACTIVITY. THE BETA CHAIN IS REQUIRED

FOR INITIATION OF REPLICATION ONCE IT IS CLAMPED ONTO DNA, IT SLIDES FREELY (BIDIRECTIONAL AND ATP-INDEPENDENT) ALONG DUPLEX DNA *(BY SIMILARITY)*.
CATALYTIC ACTIVITY: N deoxynucleoside triphosphate = N diphosphate + $\{DNA\}_N$.
SUBUNIT: CONTAINS A CORE (COMPOSED OF ALPHA, EPSILON, AND THETA CHAINS) THAT CAN REPAIR SHORT GAPS CREATED BY NUCLEASE IN DUPLEX DNA. FOR EFFICIENT REPLICATION OF THE LONG, SINGLE-STRANDED TEMPLATES, POL III REQUIRES THE AUXILIARY CHAINS BETA, GAMMA, AND DELTA *(BY SIMILARITY)*.
SUBCELLULAR LOCATION: Cytoplasmic *(By similarity)*.

Copyright

Cross-references

EMBL	AP003358; BAB56164.1; -.	[EMBL / GenBank / DDBJ] [CoDingSequence]
	AP003129; BAB41218.1; -.	[EMBL / GenBank / DDBJ] [CoDingSequence]
	AP004822; BAB93867.1; -.	[EMBL / GenBank / DDBJ] [CoDingSequence]
CMR	P50029; SAV0002.	
InterPro	IPR001001; DNA_polIII_beta. Graphical view of domain structure.	
Pfam	PF00712; DNA_pol3_beta; 1. PF02767; DNA_pol3_beta_2; 1. PF02768; DNA_pol3_beta_3; 1.	
SMART	SM00480; POL3Bc; 1.	
TIGRFAMs	TIGR00663; dnan; 1.	
ProDom	[Domain structure / List of seq. sharing at least 1 domain].	
BLOCKS	P50029.	
ProtoNet	P50029.	
ProtoMap	P50029.	
PRESAGE	P50029.	
DIP	P50029.	
ModBase	P50029.	
SWISS-2DPAGE	Get region on 2D PAGE.	

Keywords

Transferase; **DNA-directed DNA polymerase**; **DNA replication**; **Complete proteome**.

Features

None

Sequence information

Length: **377 AA**	Molecular weight: **41913 Da**	CRC64: **0A985EF94E044FBC** [This is a checksum on the sequence]

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        10          20          30          40          50          60
         |           |           |           |           |           |
MMEFTIKRDY FITQLNDTLK AISPRTTLPI LTGIKIDAKE HEVILTGSDS EISIEITIPK

        70          80          90         100         110         120
         |           |           |           |           |           |
TVDGEDIVNI SETGSVVLPG RFFVDIIKKL PGKDVKLSTN EQFQTLITSG HSEFNLSGLD

       130         140         150         160         170         180
         |           |           |           |           |           |
PDQYPLLPQV SRDDAIQLSV KVLKNVIAQT NFAVSTSETR PVLTGVNWLI QENELICTAT

       190         200         210         220         230         240
         |           |           |           |           |           |
DSHRLAVRKL QLEDVSENKN VIIPGKALAE LNKIMSDNEE DIDIFFASNQ VLFKVGNVNF

       250         260         270         280         290         300
         |           |           |           |           |           |
ISRLLEGHYP DTTRLFPENY EIKLSIDNGE FYHAIDRASL LAREGGNNVI KLSTGDDVVE

       310         320         330         340         350         360
         |           |           |           |           |           |
LSSTSPEIGT VKEEVDANDV EGGSLKISFN SKYMMDALKA IDNDEVEVEF FGTMKPFILK

       370
         |
PKGDDSVTQL ILPIRTY
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P50029 in FASTA format

View entry in original SWISS-PROT format
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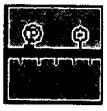
Direct BLAST submission at EMBnet-CH/SIB (Switzerland)

Direct BLAST submission at NCBI (Bethesda, USA)

ScanProsite, MotifScan

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)



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L4: Entry 3 of 16 File: USPT Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287844 B1
TITLE: Compositions and methods for controlling genetically engineered organisms

Brief Summary Text (15):
It is not intended that the present invention be limited to particular polymerases or promoters. In one embodiment, the bacteriophage T7 RNA polymerase is used and the microorganism expresses the polymerase inhibitor, T7 lysozyme.

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L21: Entry 32 of 211 File: USPT Oct 16, 2001

DOCUMENT-IDENTIFIER: US 6303568 B1
TITLE: Therapeutic antimicrobial polypeptides, their use and methods for preparation

Brief Summary Text (11):
The present invention provides for a method for treatment of an animal having a bacterial infection caused by either or both gram-positive or gram-negative bacteria, such as by a member of the group consisting of Brucella, Listeria, Pseudomonas (other than P. solanacium), Staphylococcus or a protozoan infection caused by a member of the group consisting of Trypanosoma and Plasmodia, which method comprises administration to said mammal of an antibacterial amount of an antimicrobial polypeptide selected from the group consisting of a cecropin, an attacin, a lysozyme, a polypeptide transcribed from gene 13 of phage P22, an S protein from lambda phage, and an E protein from phage PhiX174. As another aspect of the present invention, there is provided a biosynthetic method for producing the antimicrobial polypeptides of the present invention which method includes the steps of

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L6: Entry 80 of 120 File: USPT Sep 22, 1998

DOCUMENT-IDENTIFIER: US 5811654 A
TITLE: Plants genetically enhanced for nutritional quality

Brief Summary Text (37):
The antimicrobial genes in the plants of the present invention generally encode for antibacterial and/or antifungal polypeptides, and/or antiviral agents such as micRNA, not normally found in the particular plant species. Suitable antimicrobial polypeptides are, for example, derived from insect hemolymph, such as attacin. A preferred class of antimicrobial polypeptides include the lytic peptides. Exemplary lytic peptides include lysozymes, cecropins, attacins, melittins, magainins, bombinins, xenopsins, caeruleins, the polypeptide from gene 13 of phage P22, S protein from lambda phage, E protein from phage PhiX174, and the like. However, lytic peptides such as the melittins, bombinins, and magainins are generally relatively high in lytic activity, and are therefore less preferred since host plant cells may be adversely affected thereby.

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L6: Entry 119 of 120 File: USPT Mar 13, 1984

DOCUMENT-IDENTIFIER: US 4436815 A
TITLE: Method for stabilizing and selecting recombinant DNA containing host cells

Brief Summary Text (25):
The interaction of bacteriophage .lambda. with E. coli K12 is employed to illustrate the applicability of cell suicide for maintaining and stabilizing recombinant DNA host cells. Bacteriophage .lambda. is a temperate bacteriophage that follows either of two mutually exclusive cycles when infecting E. coli K12. In the lytic phase the bacteriophage DNA replicates autonomously, directs synthesis and assembly of bacteriophage components, and kills the cells concommitant with the release of mature bacteriophage. In the lysogenic phase the bacteriophage is integrated into the host's chromosome as a prophage, replicates as a marker on the chromosome, and blocks synthesis of bacteriophage components. A bacteriophage gene, .lambda.cI, codes for a repressor that maintains the lysogenic state and blocks expression of genes for bacteriophage components and maturation. If the repressor is inactivated or removed from the cell, the prophage educts from the chromosome, enters the lytic cycle, and kills the cell. Bacteriophage with a defective .lambda.cI gene cannot maintain the lysogenic state and are lethal to the cell unless a functional repressor is provided from an alternate source. In an illustrative embodiment of the present invention, .lambda.cI90 is employed as a repressor dependent prophage and a cI gene, contained in a restriction fragment and cloned into a recombinant DNA cloning vector, serves as the functional repressor.

Brief Summary Text (29):
The cloning of the .about.1.3 kb EcoRI-BamHI trp E-insulin A chain gene containing restriction fragment of plasmid pIA7.DELTA.4.DELTA.1 onto the .about.4.7 kb EcoRI-BamHI restriction fragment of plasmid pPR12, hereinafter designated pPR12.DELTA.2, results in the novel plasmid pPR17. The plasmid pIA7.DELTA.4.DELTA.1 .about.1.3 kb EcoRI-BamHI restriction fragment contains part of .DELTA.2 so therefore the construction restores .DELTA.2 to .DELTA.1. Plasmid pPR17 contains the .about.0.9 kb PstI-HincII restriction fragment of bacteriophage .lambda.cI857 and thus blocks the lytic development of bacteriophage lambda in lysogenized host cells. In addition, plasmid pPR17 codes for and expresses the aforementioned trp E-insulin A chain fused gene product at levels significantly above that of other .lambda.cI gene containing plasmids known in the art. A restriction site and functional map of plasmid pPR17 is presented in FIG. 5 of the accompanying drawings.

Brief Summary Text (32):
The cloning of the .about.1.3 kb EcoRI-BamHI trp E-insulin B chain gene containing restriction fragment of plasmid pIB7.DELTA.4.DELTA.1 onto the .about.4.7 kb EcoRI-BamHI restriction fragment of plasmid pPR12 results in the novel plasmid pPR18. Plasmid pPR18 contains the .about.0.9 kb PstI-HincII restriction fragment of bacteriophage .lambda.cI857 and thus blocks the lytic development of bacteriophage lambda in lysogenized host cells. In addition, plasmid pPR18 codes for and expresses the aforementioned trp E-insulin B chain fused

Search in SWISS-PROT and TrEMBL for: dnan

SWISS-PROT Release 40.38 of 13-Dec-2002
TrEMBL Release 22.6 of 13-Dec-2002

Number of sequences found in SWISS-PROT(46) and TrEMBL(53): **99**

Note that the selected sequences can be saved to a file to be later retrieved; to do so, go to the bottom of this page.

For more directed searches, you can use the Sequence Retrieval System SRS.

Search in SWISS-PROT: There are matches to 46 out of 120607 entries

DP3B_ACTPL (**P24701**)

DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} - Actinobacillus pleuropneumoniae (Haemophilus pleuropneumoniae)

DP3B_AQUAE (**O67725**)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR

AQ_1882} - Aquifex aeolicus

DP3B_BACHD (**Q9RCA1**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR BH0002} - Bacillus halodurans

DP3B_BACSU (**P05649**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR DNAG} - Bacillus subtilis

DP3B_BORBU (**P33761**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR BB0438} - Borrelia burgdorferi (Lyme disease spirochete)

DP3B_BUCAI (**P57127**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR BU011} - Buchnera aphidicola (subsp. Acyrthosiphon pisum) (Acyrthosiphon pisum symbiotic bacterium)

DP3B_BUCAP (**P29439**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR BUSG011} - Buchnera aphidicola (subsp. Schizaphis graminum)

DP3B_BUCRP (**Q9EVE4**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - Buchnera aphidicola (subsp. Rhopalosiphum padi)

DP3B_CAUCR (**P48198**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR CC0156} - Caulobacter crescentus

DP3B_CHLMU (**Q9PKW4**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR TC0347} - Chlamydia muridarum

DP3B_CHLPN (**Q9Z8K0**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR CPN0338 OR CP0419} - Chlamydia pneumoniae (Chlamydophila pneumoniae)

DP3B_CHLTR (**O84078**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR CT075} - Chlamydia trachomatis

DP3B_ECOLI (**P00583**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR B3701 OR Z5192 OR ECS4636} - Escherichia coli, Escherichia coli O157:H7

DP3B_HAEIN (**P43744**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR HI0992} - Haemophilus influenzae
DP3B_HELPJ (**Q9ZLX4**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR JHP0452} - Helicobacter pylori J99 (Campylobacter pylori J99)
DP3B_HELPY (**O25242**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR HP0500} - Helicobacter pylori (Campylobacter pylori)
DP3B_LACLA (**Q9CJJ1**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR LL0002} - Lactococcus lactis (subsp. lactis) (Streptococcus lactis)
DP3B_LACLC (**O54376**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - Lactococcus lactis (subsp. cremoris) (Streptococcus cremoris)
DP3B_MICLU (**P21174**)
DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} - Micrococcus luteus (Micrococcus lysodeikticus)
DP3B_MYCBO (**O33914**)
DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} - Mycobacterium bovis
DP3B_MYCCA (**P24117**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - Mycoplasma capricolum
DP3B_MYCGE (**P47247**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR MG001} - Mycoplasma genitalium
DP3B_MYCLE (**P46387**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR ML0002} - Mycobacterium leprae
DP3B_MYCPA (**Q9L7L6**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - Mycobacterium paratuberculosis
DP3B_MYCPN (**Q50313**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR MPN001

OR MP153} - Mycoplasma pneumoniae

DP3B_MYCPU (**Q98RK6**)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR MYPU_0020} - Mycoplasma pulmonis

DP3B_MYCSM (**P52851**)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - Mycobacterium smegmatis

DP3B_MYCTU (**Q50790**)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR RV0002 OR MT0002 OR MTV029.02 OR MTCY10H4.0} - Mycobacterium tuberculosis

DP3B_PROMI (**P22838**)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - Proteus mirabilis

DP3B_PSEAE (**Q9I7C4**)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR PA0002} - Pseudomonas aeruginosa

DP3B_PSEPU (**P13455**)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - Pseudomonas putida

DP3B_RHOCA (**P31861**)

DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} - Rhodobacter capsulatus (Rhodopseudomonas capsulata)

DP3B_RICCN (**Q92I37**)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR RC0583} - Rickettsia conorii

DP3B_RICPR (**Q9ZDB3**)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR RP419} - Rickettsia prowazekii

DP3B_SALTY (**P26464**)

DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR STM3837} - Salmonella typhimurium

DP3B_SERMA (**P29438**)

DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} - Serratia marcescens

Search in TrEMBL: There are matches to 53 out of 729579 entries

Q50381
DnaN protein (Fragment) {GENE:DNAN} - Mycobacterium smegmatis
Q8K8Z9
Beta subunit of DNA polymerase III {GENE:DNAN OR SPYM3_0002} - Streptococcus pyogenes (serotype M3)
Q8KGG7
DNA polymerase III, beta subunit {GENE:DNAN OR CT0001} - Chlorobium tepidum
Q8KQT1
DNA polymerase III beta chain {GENE:DNAN} - Xanthomonas campestris (pv. campestris)
Q8P329
Beta subunit of DNA polymerase III {GENE:DNAN OR SPYM18_0002} - Streptococcus pyogenes (serotype M18)
Q8PEH4
DNA polymerase III beta chain {GENE:DNAN OR XCC0002} - Xanthomonas campestris (pv. campestris)
Q8PRG1
DNA polymerase III beta chain {GENE:DNAN OR XAC0002} - Xanthomonas axonopodis (pv. citri)
Q8RDL5
DNA polymerase III beta subunit {GENE:DNAN OR TTE0002} - Thermoanaerobacter tengcongensis
Q8RJH9
DNA polymerase III subunit protein (Fragment) {GENE:DNAN-LIKE} - Streptomyces caespitosus
Q8RNR9
DNA polymerase III (Fragment) {GENE:DNAN} - Streptomyces avermitilis
Q8UIJ4
DNA polymerase III, beta chain {GENE:DNAN OR ATU0301 OR AGR_C_520} - Agrobacterium tumefaciens (strain C58 / ATCC 33970)

Q8XPG1
DNA-directed DNA polymerase III beta chain {GENE:DNAN OR CPE0002} - Clostridium perfringens
Q8XTV5
Probable DNA polymerase III (Beta chain) protein (EC 2.7.7.7) {GENE:DNAN OR RSC3441 OR RS01822} - Ralstonia solanacearum (Pseudomonas solanacearum)
Q8YAW1
DNA polymerase III, beta chain {GENE:DNAN OR LMO0002} - Listeria monocytogenes
Q8YVG8
DNA polymerase III beta subunit {GENE:DNAN OR ALR2010} - Anabaena sp. (strain PCC 7120)
Q8Z9U8
DNA polymerase III, beta subunit protein (EC 2.7.7.7) {GENE:DNAN OR YPO4096} - Yersinia pestis
Q92FV1
DNA polymerase III, beta chain {GENE:DNAN OR LIN0002} - Listeria innocua
Q92SN6
Probable DNA polymerase III, beta chain protein (EC 2.7.7.7) {GENE:DNAN OR R00335 OR SMC00415} - Rhizobium meliloti (Sinorhizobium meliloti)
Q93LM9
DNA polymerase III beta subunit DnaN {GENE:DNAN} - Cytophaga johnsonae
Q9A209
Beta subunit of DNA polymerase III (EC 2.7.7.7) {GENE:DNAN OR SPY0003} - Streptococcus pyogenes
Q9AD31
Putative DNA-polymerase III, beta chain {GENE:DNAN OR SCP1.119} - Streptomyces coelicolor [Plasmid SCP1]
Q9AJ55
DnaN {GENE:DNAN} - Buchnera aphidicola
Q9AJ56

DnaN {GENE:DNAN} - Buchnera aphidicola
Q9AJ57
DnaN {GENE:DNAN} - Buchnera aphidicola
Q9AJ58
DnaN {GENE:DNAN} - Buchnera aphidicola
Q9AJ59
DnaN {GENE:DNAN} - Buchnera aphidicola
Q9CLQ5
DnaN {GENE:DNAN OR PM1160} - Pasteurella multocida
Q9EVD9
DNA polymerase III beta subunit (Fragment) {GENE:DNAN} - Rhizobium meliloti (Sinorhizobium meliloti)
Q9EVE6
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVE7
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVE8
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVE9
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVF0
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
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DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVF2
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVF3
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVF4
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVF5
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVF6
DNA polymerase III beta subunit (DnaN) {GENE:DNAN} - Buchnera aphidicola

Q9EVF7
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVF8
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9EVN6
DnaN {GENE:DNAN} - Mycobacterium tuberculosis
Q9EVR1
DNA polymerase III beta subunit (Fragment) {GENE:DNAN} - Streptococcus pyogenes
Q9JW44
Putative DNA polymerase III, beta subunit (EC 2.7.7.7) {GENE:DNAN OR NMA0553} - Neisseria meningitidis (serogroup A)
Q9PJA9
DNA polymerase III, beta chain (EC 2.7.7.7) {GENE:DNAN OR CJ0002} - Campylobacter jejuni
Q9PR66
DNA pol III beta chain {GENE:DNAN OR UU079} - Ureaplasma parvum (Ureaplasma urealyticum biotype 1)
Q9REM9
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9REN0
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9REN1
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9REN2
DNA polymerase III beta subunit {GENE:DNAN} - Buchnera aphidicola
Q9RNB9
DNA polymerase III beta subunit (EC 2.7.7.7) (Fragment) {GENE:DNAN} - Microcystis aeruginosa
Q9X9D6
Putative DnaN protein (Fragment) {GENE:DNAN} - Thermus thermophilus
Q9ZB90
DnaN (Fragment) {GENE:DNAN} - Mycobacterium avium

New Search

in SWISS-PROT/TrEMBL by AC, ID, description, gene name, organism

Please do NOT use any boolean operators (and, or, etc.)

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TIGR
THE INSTITUTE FOR GENOMIC RESEARCH

tigr fams
tigr protein families

HMM Profile Page
Accession #: TIGR00663 Name: dnan

Both TIGRFAMs and Pfams are displayed on this page. TIGRFAMs and Pfams
based on Hidden Markov Models or HMMs. An HMM is a statistical model for
system that can be represented as a succession of transitions between discre
states. Scores are reported both in bits of information and as an E-value. See
for more information on this TIGRFAM or Pfam and its HMM.

---

dnan Information: See below for detailed information on this family, includin
cutoff score for inclusion in this family and the average score of genes/prote
this family. To view all genes with the same EC number, click on the **EC Numb**
To view more information on the Role Category for this family, click on the **Ro**
Category link.

Accessi n:	TIGR00663	**Name:** dnan	**Is logy Type:** equi
Common Name:	DNA polymerase III, beta subunit		
Noise Cutoff:	0.00	**Trusted Cutoff:** 30.00	**Avg. Score:** 531. 27.0
EC Number:	2.7.7.7	**HMM length:** 406	
Relationship:	**InterPro assignment:** IPR001001		
Role Category:	**Mainrole:** DNA metabolism	**Subrole:** DNA replication, recombination, and r	

Gene Ontology (GO) Terms:		
GO:0003887	function	DNA-directed DNA polymer
GO:0006260	process	DNA replication
GO:0009360	component	DNA polymerase III comple

Author(s):	Loftus BJ, Eisen JA	**Created:** Dec 8 1999 12:19PM	**Last Modified:** May 2:32

References: EGAD: 28015
SWISSPROT: P43744
SWISSPROT: P00583
MUID: 84237568 .

Comments: All proteins in this family for which functions are known a components of the DNA polymerase III complex (beta sul This family is based on the phylogenomic analysis of JA Ei (1999, Ph.D. Thesis, Stanford University).

Display Hits and Overlaps: To view all CMR proteins that are members of thi click on **All CMR Hits**. To display any overlapping HMMs, click on **Any overlapp HMMs?**.

All CMR Hits to TIGR00663 | Any Overlapping HMMs?

Alignment Display: View a multiple protein alignment display for this HMM. C
view the HMM alignment either in **FASTA** or **MSF** format and then depress t
submit button below. Depress the **JalView** button to start a protein alignmen
program. This program allows the user to view the alignment in different way
highlight the identical and similar amino acids in the alignment).

MSF HMM Alignment ▼ Submit

Members of the dnan HMM

Listed below are the current members of this HMM, including those not found
CMR. Click on the **Protein ID** link to view the protein report for a particular m
of this family.

PROTEIN ID	COORDINATES	DATABASE
CMR: TP0002	1-370	TIGR - CMR
CMR: HI0992	1-366	TIGR - CMR
CMR: NTL01SS00293	1-389	TIGR - CMR
CMR: NTL01BS00002	1-377	TIGR - CMR
CMR: NTL01ML0004	13-397	TIGR - CMR
CMR: BB0438	5-384	TIGR - CMR
SP: P33761	5-384	SWISS-PROT/TrE
CMR: HP0500	1-374	TIGR - CMR
SP: P29439	1-366	SWISS-PROT/TrE

CMR: NTL01HP00448	1-374	TIGR - CMR
CMR: NTL01PM1162	1-366	TIGR - CMR
SP: P34029	1-362	SWISS-PROT/TrE
SP: Q9L7L6	13-397	SWISS-PROT/TrE
CMR: NTL02MT00002	13-400	TIGR - CMR
GP: 9845532	13-400	GenBank
GP: 11320921	1-366	GenBank
SP: P22838	1-367	SWISS-PROT/TrE
CMR: NTL03EC4650	1-366	TIGR - CMR
SP: P52851	11-395	SWISS-PROT/TrE
GP: 16504793	1-366	GenBank
CMR: NTL01ST3835	1-366	TIGR - CMR
GP: 15988190	1-366	GenBank
GP: 15981986	1-366	GenBank
GP: 1321905	13-397	GenBank
CMR: NTL03PA00003	1-367	TIGR - CMR
SP: P13455	1-367	SWISS-PROT/TrE
CMR: VC0013	1-366	TIGR - CMR
GP: 16412423	1-380	GenBank
GP: 16409361	1-380	GenBank
CMR: NTL02SA0003	2-376	TIGR - CMR
CMR: NTL01BH0003	1-379	TIGR - CMR
SP: P52023	1-373	SWISS-PROT/TrE
GP: 6606556	1-366	GenBank
CMR: NTL01BA00012	1-366	TIGR - CMR
CMR: NTL01XF00003	1-366	TIGR - CMR
GP: 6606558	1-366	GenBank

GP: 11320919	1-366	GenBank
CMR: EF0002	1-375	TIGR - CMR
GP: 17131100	1-385	GenBank
GP: 6606560	1-366	GenBank
GP: 11320917	1-367	GenBank
GP: 6606554	1-366	GenBank
GP: 11320911	1-368	GenBank
GP: 11320909	1-368	GenBank
GP: 11320907	1-367	GenBank
GP: 11320913	1-367	GenBank
GP: 11320899	1-367	GenBank
GP: 11320915	1-368	GenBank
GP: 15022819	1-363	GenBank
GP: 11320905	1-367	GenBank
GP: 11320903	1-367	GenBank
GP: 17430465	2-371	GenBank
GP: 13398078	1-368	GenBank
GP: 13398088	1-368	GenBank
GP: 13398101	1-368	GenBank
GP: 13398095	1-368	GenBank
GP: 11320897	1-368	GenBank
SP: P27903	1-374	SWISS-PROT/TrE
GP: 13398084	1-368	GenBank
GP: 11320893	1-368	GenBank
GP: 17983990	26-397	GenBank
GP: 11320895	1-368	GenBank
EGAD:24168	1-374	TIGR - EGAD

CMR: NTL01NM00516	2-367	TIGR - CMR
CMR: SP0002	2-377	TIGR - CMR
CMR: CC0156	1-372	TIGR - CMR
GP: 11320901	1-367	GenBank
CMR: NTL02SP0002	2-377	TIGR - CMR
CMR: NMB1902	2-367	TIGR - CMR
CMR: NTL02ML4325	1-372	TIGR - CMR
CMR: NT01MC2948	1-376	TIGR - CMR
GP: 17738634	1-372	GenBank
GP: 15155199	32-403	GenBank
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CMR: TM0262	1-365	TIGR - CMR
CMR: NTL01SPL0005	2-377	TIGR - CMR
CMR: TC0347	1-363	TIGR - CMR
CMR: NTL01CT00076	51-413	TIGR - CMR
SP: O84078	51-413	SWISS-PROT/TrE
GP: 16885215	2-377	GenBank
CMR: NTL01LL0003	2-379	TIGR - CMR
CMR: CP0419	1-363	TIGR - CMR
CMR: NTL02CP00339	1-363	TIGR - CMR
CMR: NTL01CP00329	1-363	TIGR - CMR
SP: O54376	2-379	SWISS-PROT/TrE
GP: 13620601	1-370	GenBank
GP: 14531036	1-370	GenBank
CMR: NTL01AA01298	1-363	TIGR - CMR
GP: 17134654	1-373	GenBank
GP: 15619665	2-379	GenBank

CMR: NTL01CJ00004	1-355	TIGR - CMR
CMR: NTL01RP00403	2-381	TIGR - CMR
SP: P24117	1-373	SWISS-PROT/TrE
CMR: DR0001	2-372	TIGR - CMR
GP: 7716426	1-365	GenBank
SP: P21174	1-309	SWISS-PROT/TrE
EGAD:24146	1-309	TIGR - EGAD
CMR: PG1853	1-374	TIGR - CMR
CMR: NTL01MP00147	1-380	TIGR - CMR
CMR: NTL02MP0003	1-372	TIGR - CMR
GP: 3953450	13-258	GenBank
CMR: NTL01UU00079	1-379	TIGR - CMR
CMR: MG001	1-364	TIGR - CMR
GP: 6468432	12-384	GenBank

Questions? Comments? Please feel free to send us feedback!

InterPro

Sequence Search
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InterPro - Protein P50029

Protein	Match Display
SWISS-PROT DP3B_STAAM P50029	IPR001001 PF00712
	IPR001001 PF02767
	IPR001001 PF02768
	IPR001001 SM00480
	IPR001001 TIGR00663

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L6: Entry 6 of 120 File: USPT Aug 13, 2002

US-PAT-NO: 6432444
DOCUMENT-IDENTIFIER: US 6432444 B1

TITLE: Use of bacterial phage associated lysing enzymes for treating dermatological infections

DATE-ISSUED: August 13, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fischetti; Vincent	West Hempstead	NY		
Loomis; Lawrence	Columbia	MD		

US-CL-CURRENT: 424/443; 424/447, 424/45, 424/450, 424/78.03, 424/78.05, 424/78.06, 424/78.07, 424/94.1, 514/2, 514/937, 514/944, 514/948

CLAIMS:

What we claim is:

1. A bandage for treating a bacterial infection of skin, wherein said bandage contains a composition produced by the method of: (a) obtaining an effective amount of at least one lytic enzyme genetically coded for by a specific bacteriophage specific for a bacteria infecting said skin, wherein the bacteria to be treated is selected from the group consisting of Staphylococcus, Pseudomonas, Streptococcus, and combinations thereof, wherein said at least one said lytic enzyme is specific for and has the ability to digest a cell wall of one of said bacteria and is coded for by the same said bacteriophage capable of infecting said bacteria being digested; and (b) mixing said at least one lytic enzyme produced in step (a) with a topical carrier selected from the group consisting of an ointment, a cream, an alcohol based liquid, an aqueous liquid, a water soluble gel, a lotion, a non-aqueous liquid base, a mineral oil base, a blend of mineral oil and petrolatum, lanolin, liposomes, protein carriers, a powdered cellulose carmel, and combinations thereof.

2. The composition according to claim 1, further comprising at least one preservative.

3. The composition according to claim 2, wherein said preservative is a bacteriocidal agent or bacteriostatic agent.

4. The composition according to claim 1, further comprising a surfactant in an amount effective to potentiate a therapeutic effect of the composition.

5. The composition according to claim 1, further comprising lystostaphin for the

treatment of Staphylococcus aureus.

6. The composition according to claim 1, further comprising a lysozyme.

7. The composition according to claim 1, further comprising at least one antioxidant.

8. The composition according to claim 1, further comprising at least one anti-inflammatory agent.

9. The composition according to claim 1, further comprising an antibiotic.

Entry name	**DP3B_STAAM**
Primary accession number	**P50029**
Secondary accession numbers	None
Entered in SWISS-PROT in	Release 34, October 1996
Sequence was last modified in	Release 34, October 1996
Annotations were last modified in	Release 41, June 2002
Name and origin of the protein	
Protein name	**DNA polymerase III, beta chain**
Synonym	EC 2.7.7.7
Gene name	**DNAN** or **SAV0002** or **SA0002** or **MW0002**
From	Staphylococcus aureus (strain Mu50 / ATCC 700699) [TaxID: 158878] Staphylococcus aureus (strain N315) [TaxID: 158879] Staphylococcus aureus (strain MW2) [TaxID: 196620] Staphylococcus aureus [TaxID: 1280]
Taxonomy	Bacteria; Firmicutes; Bacillales; Staphylococcus.
References	

[1]	SEQUENCE FROM NUCLEIC ACID. **STRAIN**=Mu50 / ATCC 700699, and N315; MEDLINE=21311952; PubMed=11418146; [NCBI, ExPASy, EBI, Israel, Japan] Kuroda M., Ohta T., Uchiyama I., Baba T., Yuzawa H., Kobayashi I., Cui L., Oguchi A., Aoki K.-I., Nagai Y., Lian J.-Q., Ito T., Kanamori M., Matsumaru H., Maruyama A., Murakami H., Hosoyama A., Mizutani-Ui Y., Takahashi N.K., Sawano T., Inoue R.-I., Kaito C., Sekimizu K., Hirakawa H., Kuhara S., Goto S., Yabuzaki J., Kanehisa M., Yamashita A., Oshima K., Furuya K., Yoshino C., Shiba T., Hattori M., Ogasawara N., Hayashi H., Hiramatsu K.; "Whole genome sequencing of meticillin-resistant Staphylococcus aureus."; Lancet 357:1225-1240(2001).
[2]	SEQUENCE FROM NUCLEIC ACID. **STRAIN**=MW2; MEDLINE=22040717; PubMed=12044378; [NCBI, ExPASy, EBI, Israel, Japan] Baba T., Takeuchi F., Kuroda M., Yuzawa H., Aoki K.-I., Oguchi A., Nagai Y., Iwama N., Asano K., Naimi T., Kuroda H., Cui L., Yamamoto K., Hiramatsu K.; "Genome and virulence determinants of high virulence community-acquired MRSA."; Lancet 359:1819-1827(2002).
[3]	SEQUENCE FROM NUCLEIC ACID. **STRAIN**=YB886; MEDLINE=95206242; PubMed=7898435; [NCBI, ExPASy, EBI, Israel, Japan] Alonso J.C., Fisher L.M.; "Nucleotide sequence of the recF gene cluster from Staphylococcus aureus and complementation analysis in Bacillus subtilis recF mutants."; Mol. Gen. Genet. 246:680-686(1995).

Comments

FUNCTION: DNA POLYMERASE III IS A COMPLEX, MULTICHAIN ENZYME RESPONSIBLE FOR MOST OF THE REPLICATIVE SYNTHESIS IN BACTERIA. THIS DNA POLYMERASE ALSO EXHIBITS 3' TO 5' EXONUCLEASE ACTIVITY. THE BETA CHAIN IS REQUIRED

FOR INITIATION OF REPLICATION ONCE IT IS CLAMPED ONTO DNA, IT SLIDES FREELY (BIDIRECTIONAL AND ATP-INDEPENDENT) ALONG DUPLEX DNA *(BY SIMILARITY)*.
CATALYTIC ACTIVITY: N deoxynucleoside triphosphate = N diphosphate + $\{DNA\}_N$.
SUBUNIT: CONTAINS A CORE (COMPOSED OF ALPHA, EPSILON, AND THETA CHAINS) THAT CAN REPAIR SHORT GAPS CREATED BY NUCLEASE IN DUPLEX DNA. FOR EFFICIENT REPLICATION OF THE LONG, SINGLE-STRANDED TEMPLATES, POL III REQUIRES THE AUXILIARY CHAINS BETA, GAMMA, AND DELTA *(BY SIMILARITY)*.
SUBCELLULAR LOCATION: Cytoplasmic *(By similarity)*.

Copyright

Cross-references

EMBL	AP003358; BAB56164.1; -.	[EMBL / GenBank / DDBJ] [CoDingSequence]
	AP003129; BAB41218.1; -.	[EMBL / GenBank / DDBJ] [CoDingSequence]
	AP004822; BAB93867.1; -.	[EMBL / GenBank / DDBJ] [CoDingSequence]
CMR	P50029; SAV0002.	
InterPro	IPR001001; DNA_polIII_beta. Graphical view of domain structure.	
Pfam	PF00712; DNA_pol3_beta; 1. PF02767; DNA_pol3_beta_2; 1. PF02768; DNA_pol3_beta_3; 1.	
SMART	SM00480; POL3Bc; 1.	
TIGRFAMs	TIGR00663; dnan; 1.	
ProDom	[Domain structure / List of seq. sharing at least 1 domain].	
BLOCKS	P50029.	
ProtoNet	P50029.	
ProtoMap	P50029.	
PRESAGE	P50029.	
DIP	P50029.	
ModBase	P50029.	
SWISS-2DPAGE	Get region on 2D PAGE.	

Keywords

Transferase; DNA-directed DNA polymerase; DNA replication; Complete proteome.

Features

None

Sequence information

Length: **377 AA**	Molecular weight: **41913 Da**	CRC64: **0A985EF94E044FBC** [This is a checksum on the sequence]

```
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          |           |           |           |           |           |
MMEFTIKRDY FITQLNDTLK AISPRTTLPI LTGIKIDAKE HEVILTGSDS EISIEITIPK

         70          80          90         100         110         120
          |           |           |           |           |           |
TVDGEDIVNI SETGSVVLPG RFFVDIIKKL PGKDVKLSTN EQFQTLITSG HSEFNLSGLD

        130         140         150         160         170         180
          |           |           |           |           |           |
PDQYPLLPQV SRDDAIQLSV KVLKNVIAQT NFAVSTSETR PVLTGVNWLI QENELICTAT

        190         200         210         220         230         240
          |           |           |           |           |           |
DSHRLAVRKL QLEDVSENKN VIIPGKALAE LNKIMSDNEE DIDIFFASNQ VLFKVGNVNF

        250         260         270         280         290         300
          |           |           |           |           |           |
ISRLLEGHYP DTTRLFPENY EIKLSIDNGE FYHAIDRASL LAREGGNNVI KLSTGDDVVE

        310         320         330         340         350         360
          |           |           |           |           |           |
LSSTSPEIGT VKEEVDANDV EGGSLKISFN SKYMMDALKA IDNDEVEVEF FGTMKPFILK

        370
          |
PKGDDSVTQL ILPIRTY
```

P50029 in FASTA format

View entry in original SWISS-PROT format
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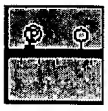
Direct BLAST submission at EMBnet-CH/SIB (Switzerland)

Direct BLAST submission at NCBI (Bethesda, USA)

ScanProsite, MotifScan

Tools

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)



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L14: Entry 1 of 7 File: USPT Nov 26, 2002

DOCUMENT-IDENTIFIER: US 6485902 B2
TITLE: Use of bacteriophages for control of escherichia coli O157

Other Reference Publication (5):
J. Alisky, K. Iczkowski, A. Rapoport and N. Troitsky, 1998, "Bacteriophages show promise as antimicrobial agents", Journal of Infection 36: 5-15.

Other Reference Publication (13):
Alexander Sulakvelidze, Zemphira Alavidze and J. Glenn Morris Jr., Mar. 2001, "Bacteriophage Therapy", Antimicrobial Agents and Chemotherapy 45: 649-659.

gene product at levels significantly above that of other .lambda.cI gene containing plasmids known in the art. A restriction site and functional map of plasmid pPR18 is presented in FIG. 6 of the accompanying drawings.

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L4: Entry 1 of 7 File: USPT Aug 13, 2002

DOCUMENT-IDENTIFIER: US 6432444 B1
TITLE: Use of bacterial phage associated lysing enzymes for treating dermatological infections

Other Reference Publication (14):
Loessner, et al. (1999) "Evidence for a holin-like protein gene fully embedded out of frame in the endolysin gene of Staphylococcus aureus Bacteriophage." (1999) Journal of Bacteriology, 181(15) p. 4452-4460.

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L3: Entry 4 of 18 File: USPT Aug 13, 2002

DOCUMENT-IDENTIFIER: US 6432444 B1
TITLE: Use of bacterial phage associated lysing enzymes for treating dermatological infections

Other Reference Publication (14):
Loessner, et al. (1999) "Evidence for a holin-like protein gene fully embedded out of frame in the endolysin gene of Staphylococcus aureus Bacteriophage." (1999) Journal of Bacteriology, 181(15) p. 4452-4460.

Other Reference Publication (17):
Oki Masaya et al. (1997) "Functional and structural features of the holin HOL protein of the Lactobacillus plantarum phage phi-gle: Analysis in Escherichia coli system." Gene (AMSTERDAM) 197(1-2) p 137-145.

DP3B_SPICI (**P34029**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - Spiroplasma citri

DP3B_STAAM (**P50029**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR SAV0002 OR SA0002 OR MW0002} - Staphylococcus aureus (strain Mu50 / ATCC 700699), Staphylococcus aureus (strain N315), Staphylococcus aureus (strain MW2), Staphylococcus aureus

DP3B_STRCO (**P27903**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR SCO3878 OR SCH18.15C} - Streptomyces coelicolor

DP3B_STRPN (**O06672**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR SP0002} - Streptococcus pneumoniae

DP3B_SYNP7 (**P52023**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN} - Synechococcus sp. (strain PCC 7942) (Anacystis nidulans R2)

DP3B_SYNY3 (**P72856**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR SLR0965} - Synechocystis sp. (strain PCC 6803)

DP3B_TREPA (**O83048**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR TP0002} - Treponema pallidum

DP3B_VIBCH (**Q9KVX5**)
DNA polymerase III, beta chain (EC 2.7.7.7). {GENE: DNAN OR VC0013} - Vibrio cholerae

DP3B_VIBHA (**P52620**)
DNA polymerase III, beta chain (EC 2.7.7.7) (Fragment). {GENE: DNAN} - Vibrio harveyi

YRL3_MYCCA (**P43041**)
Hypothetical 20.7 kDa protein in KSGA-DNAN intergenic region (ORF L3). - Mycoplasma capricolum

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L1: Entry 5 of 16 File: USPT Apr 24, 2001

DOCUMENT-IDENTIFIER: US 6221642 B1
TITLE: Process for reconstituting the polymerase III* and other subassemblies of E. coli DNA polymerase III holoenzyme from peptide subunits

Brief Summary Text (8):
Within Pol II, the .alpha. subunit (dnaE) contains the DNA polymerase activity (Blanar et al., 1984, Proc. Natl Acad. Sci. USA, vol. 81, pp 46224626), and the .epsilon. subunit (dnaQ,mutD) is the proofreading 3'-5' exonuclease (Scheuetmann and Echols, 1985, Proc. Natl Acad. Sci. USA, vol. 81, pp 7747-7751; DeFrancesco et al., 1984, J. Biol. Chem, vol. 259, pp 5567-5573). The .alpha. subunit forms a tight 1:1 complex with .epsilon. (Maki and Kornberg, 1985, J. Biol. Chem., vol. 260, pp 12987-12992). Whereas most DNA polymerases have 3'-5' exonuclease activity, only the holoenzyme relegates this activity to an accessory protein. The following three accessory proteins of the holoenzyme are known to be required for DNA replication as they are products of genes that are essential for cell viability: .beta. (dnaN) (Burgers et al., 1981, Proc. Natl Acad. Sci. USA, vol. 78, pp 5391-5395), .tau., and .gamma. (the latter two both encoded by the dnaXZ gene) (Kodaira et al., 1983, Mol. Gen. Genet., vol. 192, pp 80-86).

Other Reference Publication (18):
Ohmori, H., Kimura, M., Nagata, T., and Sakakibara, Y., "Structural analysis of the dnaA and dnaN genes of Escherichia coli," Gene, 28:159-170 (1984).

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L3: Entry 13 of 18 File: USPT Jul 10, 2001

DOCUMENT-IDENTIFIER: US 6258560 B1
TITLE: Process for bacterial production of polypeptides

Detailed Description Text (5):
As used herein, "gene t" or "t gene" or "holin" refers to a lytic gene of bacteriophage T4 that is required for lysis but does not appear to have lysozyme activity. See also Molecular Genetics of Bacteriophage T4, supra, p. 398-399.

NiceProt View of TrEMBL: O80063

Printer-friendly view | Quick BlastP search

[General] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

General information about the entry	
Entry name	O80063
Primary accession number	O80063
Secondary accession numbers	None
Entered in TrEMBL in	Release 08, November 1998
Sequence was last modified in	Release 08, November 1998
Annotations were last modified in	Release 23, February 2003
Name and origin of the protein	
Protein name	**Holin**
Synonyms	None
Gene name	None
From	Staphylococcus aureus bacteriophage PVL [TaxID: 71366]
Taxonomy	Viruses; dsDNA viruses, no RNA stage; Caudovirales; Siphoviridae.
References	

[1]	SEQUENCE FROM NUCLEIC ACID. MEDLINE=98067870; PubMed=9404084; [NCBI, ExPASy, EBI, Israel, Japan] Kaneko J., Muramoto K., Kamio Y.; "Gene of LukF-PV-like component of Panton-Valentine leukocidin in Staphylococcus aureus P83 is linked with lukM."; Biosci. Biotechnol. Biochem. 61:1960-1962(1997).
[2]	SEQUENCE FROM NUCLEIC ACID. MEDLINE=98332719; PubMed=9666077; [NCBI, ExPASy, EBI, Israel, Japan] Kaneko J., Kimura T., Narita S., Tomita T., Kamio Y.; "Complete nucleotide sequence and molecular characterization of the temperate staphylococcal bacteriopbage phi PVL carrying Panton-Valentine leukocidin genes."; Gene 215:57-67(1998).

Comments

None

Cross-references

EMBL	AB009866; [EMBL / GenBank / DDBJ] BAA31897.1; -. [CoDingSequence]
InterPro	IPR006479; Holin_SPP1. Graphical view of domain structure.
Pfam	PF04688; Phage_holin; 1.
TIGRFAMs	TIGR01592; holin_SPP1; 1.
ProDom	[Domain structure / List of seq. sharing at least 1 domain].
ProtoMap	O80063.
PRESAGE	O80063.
ModBase	O80063.
SWISS-2DPAGE	Get region on 2D PAGE.

Keywords

None

Features

None

Sequence information

Length: 100 AA	Molecular weight: 11175 Da	CRC64: 8DE3F7B4EAB7E595 [This is a checksum on the sequence]

```
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         |          |          |          |          |          |
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         70         80         90        100
         |          |          |          |
WANQKLKKYK AENKYRKATG QAPIKEVMTP TNMNDTNDLG
```

O80063 in FASTA format

View entry in original TrEMBL format
View entry in raw text format (no links)
Request for annotation of this TrEMBL entry

Direct BLAST submission at EMBnet-CH/SIB (Switzerland)

Direct BLAST submission at NCBI (Bethesda, USA)

ScanProsite, MotifScan

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

Feature table viewer (Java)

Search the SWISS-MODEL Repository

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L6: Entry 104 of 120 File: USPT Aug 22, 1995

DOCUMENT-IDENTIFIER: US 5443969 A
TITLE: RNA packaging system

Detailed Description Text (41):
In a specific embodiment of the invention detailed in the examples herein, plasmids encoding and capable of expressing TMV CP and capable of transcribing OAS-linked ,DNA sequences are transformed into a host bacterial strain, referred to as E. coli BL21 (DE3) pLysE that contains a .lambda. lysogen in which the structural gene for bacteriophage T7 RNA polymerase is expressed when the inducer IPTG (isopropyl-.beta.-D-thiogalactopyranoside) is added to the medium (Studier et al., 1990, Meth. Enzymol. 185:62-89). This particular strain of bacteria also contains the plasmid vector pACYC184 which is a p15A replicon and (as pLysE) carries the T7 lysozyme structural gene. The T7 lysozyme binds to the T7 RNA polymerase and inhibits the basal level of its activity that results from transcription from the lac UV5 promoter even in the absence of the IPTG inducer. As stated supra, the lysozyme also makes the cells more fragile since it degrades the peptidoglycan wall of the bacterial cells. In this particular system, induction of T7 RNA polymerase by addition of IPTG activates expression of OAS-linked ,DNA under the control of the T7 promoter. A second expression plasmid, which expresses CP under the control of the T7 promoter also commences expression; thus, assembly of OAS-linked RNA molecules into viral particles ensues.

Detailed Description Text (58):
The host bacterial strain was E. coli BL21 (DE3). E. coli BL21 is F.sup.+, ompT, r.sub..beta..sup.-, m.beta.-. DE3 is a .lambda. derivative which carries a DNA fragment containing the lacI gene, the lac UV5 promoter, the beginning of the lacZ gene, and the gene for T7 RNA polymerase. The bacterial strain was originally provided by the Brookhaven National Research Laboratory and is a .lambda. lysogen in which the structural gene for bacteriophage T7 RNA polymerase is expressed from the lac UV5 promoter when the inducer IPTG is added to the medium (Studier et al., 1990, Meth. Enzymol. 185:62-89). The lysogenic host strain also contains the plasmid vector pACYC184 (Pouwels et al., 1985, in Cloning Vectors, Elsevier Science Publishers, Amsterdam, p. I-A-iv-9) which is a p15A replicon and, as pLysE (Studier et al., 1990, Meth. Enzymol. 185:60-89), carries the T7 lysozyme structural gene expressed from the tet promoter. The lysozyme functions to bind to the T7 RNA polymerase and inhibit the low level of constitutive transcription which occurs from the lac UV5 promoter even in the absence of the IPTG inducer. The lysozyme also makes the cells more fragile since it degrades the peptidoglycan wall of the E. coli cells.

WEST Search History

DATE: Thursday, January 02, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT; PLUR=YES; OP=AND			
L1	dna-n or dnan	16	L1
L2	pol.clm.	270	L2
L3	pol$3.clm.	93558	L3
L4	($polymerase or polymerase$).clm. same (antagon$ or inhibit$ or interact$ or block$ or inactiv$ or reduc$).clm.	273	L4
L5	L4 and ($phage or phage$ or bacteriophage or bacteriophages or bacterio-phage).clm.	19	L5
L6	(l2 or l3) and (antagon$ or inhibit$ or interact$ or block$ or inactiv$ or reduc$).clm.	19121	L6
L7	L6 and ($polymerase or polymerase$)	337	L7
L8	L7 and (subunit or sub-unit or domain or moiety)	236	L8
L9	($phage or phage$ or bacteriophage or bacteriophages or bacterio-phage)	29432	L9
L10	L9 same (antagon$ or inhibit$ or interact$ or block$ or inactiv$ or reduc$)	9022	L10
L11	L10 same (pol or pol!!! or polymerase or dnan or dna-n or $polymerase or polymerase$)	714	L11
L12	L11 and (antibiotic or anti-biotic or antimicrobial or anti-microbial)	354	L12
L13	L11 same(antibiotic or anti-biotic or antimicrobial or anti-microbial)	37	L13

L14	(pol111 or pol3 or pol-111 or pol-3 or polIII or pol-III)	28	L14
L15	$polymerase or polymerase$	29974	L15
L16	L15 same (staphylo$ or aureus)	220	L16
L17	(antagon$ or inhibit$ or interact$ or block$ or inactiv$ or reduc$)	2268326	L17
L18	L17 same l16	54	L18
L19	L18 and l9	18	L19
L20	L9 near25 (antibiotic or anti-biotic or antimicrobial or anti-microbial or l17)	4270	L20
L21	l20 same (staphylo$ or aureus or l14 or l15)	211	L21

END OF SEARCH HISTORY

WEST Search History

DATE: Thursday, January 02, 2003

Set Name sid	Query (by side)	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=AND		
L1	antimicrobial or anti-microbial or antibiotic or anti-biotic or antagonist or inhibitor or inhibits or modulator or modulation or blocks or blocker or inactivator or prevents or kills	1922046	L1
L2	L1 near5 polymerase	1629	L2
L3	L2 near10 (subunit or sub or unit or domain or beta)	75	L3
L4	l2 near3 (bacteriophage or bacterio-phage or phage or phage$)	16	L4
L5	l1 near25 (lysozyme or lytic or lysogenic or lysogenesis)	1193	L5
L6	L5 same (bacteriophage or bacterio-phage or phage or phage$)	120	L6
L7	(pelletier or gros or debow or de-bow).in. and aureus	8	L7
	DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=AND		
L8	(pelletier or gros or debow or de-bow).in. and aureus not l7	6	L8
L9	(dubow or du-bow).in. and aureus not l7	6	L9
L10	L9 not l8	0	L10

END OF SEARCH HISTORY

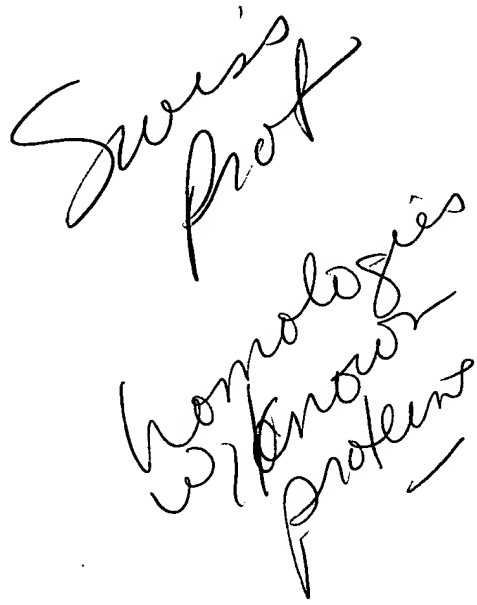
DIALOG

Set	Items	Description
S1	49	'DNA POLYMERASE BETA --ANTAGONISTS AND INHIBITO' OR 'DNA POLYMERASE BETA --DRUG EFFECTS --DE'
S2	79	'DNA POLYMERASE BETA --ANTAGONISTS AND INHIBITO'
S3	36	S2 NOT S1
S4	1	'DNA POLYMERASE BETA INHIBITOR'
S5	1	'DNA POLYMERASE III BETA SUBUNIT DNAN GENE'
S6	2	'DNA POLYMERASE III INHIBITOR'
S7	75	E28-E32
S8	1	'DNA POLYMERASE-BETA INHIBITOR'
S9	4	E46-E48
S10	1	'DNA POLYMERIZATION ASSAYS'
S11	1	'DNA POLYMERSE-BETA'
S12	86	S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11
S13	79	RD (unique items)

?t s3/9/15 16

IPB001001: DNA_polIII_beta

DNA polymerase III, beta chain



- Introduction
- Block number IPB001001A
- Block number IPB001001B
- Block number IPB001001C
- Block number IPB001001D
- Block number IPB001001E
- Block number IPB001001F
- Block number IPB001001G
- Block number IPB001001H

- InterPro entry IPR001001 (source of sequences used to make blocks)

- Block Map [About Maps]

- Logos.[About Logos]
  Select display format: [GIF] [PDF] [Postscript]

- Tree from blocks alignment. [About Trees] [About ProWeb TreeViewer]
  [Data] [ProWeb TreeViewer] [XBitmap] [GIF] [PDF] [Postscript] [Newick]

- PDB entries

- Search blocks vs other databases:
  - COBBLER sequence and BLAST searches [About COBBLER]
  - MAST Search of all blocks vs a sequence database [About MAST]
  - LAMA search of all blocks vs a blocks database [About LAMA]

- CODEHOP to design PCR primers from blocks [About CODEHOP]
- SIFT to predict amino acid substitutions in blocks [About SIFT]
- Additional Links

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Block IPB001001A

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ID   DNA_polIII_beta; BLOCK
AC   IPB001001A; distance from previous block=(4,54)
DE   DNA polymerase III, beta chain
BL   PLL;  width=49; seqs=58; 99.5%=2503; strength=1251
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DP3B_BUCAI|P57127  (   5) INNNILIKNLQKISRLLVKNTSLPILDNVLINIKNGMLSLTGTNLEIEL  20
DP3B_CAUCR|P48198  (   5) IERAALLKALGHVQSVVERRNTIPILSNILLSAEGDRLSFSATDLDMEI  31
DP3B_HAEIN|P43744  (   5) ISRENLLKPLQQVCGVLSNRPNIPVLNNVLLQIEDYRLTITGTDLEVEL  30
DP3B_MYCTU|Q50790  (  17) LLRESFADAVSWVAKNLPARPAVPVLSGVLLTGSDNGLTISGFDYEVSA  33
DP3B_PSEPU|P13455  (   5) IQREALLKPLQLVAGVVERRQTLPVLSNVLLVVQGQQLSLTGTDLEVEL  21
DP3B_RICPR|Q9ZDB3  (   6) VETKTLMQSLGFARSIIEKRNVIPEYANIKLSAQDGNLELSSTNMDLYL  79
DP3B_SPICI|P34029  (   5) IKRDKILDELLKVSRIISQKTLIPSLLGILIEVKKDKITFTTSDGDTSI  65
DP3B_STAAU|P50029  (   6) IKRDYFITQLNDTLKAISPRTTLPILTGIKIDAKEHEVILTGSDSEISI  47
DP3B_STRPN|O06672  (   6) INKNLFLQALNITKRAISSKNAIPILSTVKIDVTNEGVTLIGSNGQISI  42
DP3B_SYNY3|P72856  (   5) CRQSDLSSGLSLVSRAVSSRPTHPVLGNVLLEADADKNYLRLTAFDLSL  66
DP3B_ECOLI|P00583  (   5) VEREHLLKPLQQVSGPLGGRPTLPILGNLLLQVADGTLSLTGTDLEMEM  25
DP3B_PROMI|P22838  (   5) IEREQLLKPLQQVSGPLGGRPTLPILGNLLLKVTENTLSLTGTDLEMEM  27
DP3B_SALTY|P26464  (   5) VEREHLLKPLQQVSGPLGGRPTLPILGNLLLQVADGTLSLTGTDLEMEM  25
DP3B_AQUAE|O67725  (   5) VDREELEEVLKKARESTEKKAALPILANFLLSAKEENLIVRATDLENYL  71
DP3B_BACHD|Q9RCA1  (   5) IDRDIFVQNVNHVSKAVSSRTTIPILTGIKIVADHEGVTLTGSDSDISI  34
DP3B_BUCAP|P29439  (   5) IQNDILTKNLKKITRVLVKNISFPILENILIQVEDGTLSLTTTNLEIEL  18
DP3B_CHLMU|Q9PKW4  (   5) ISRNELGNLIKKVQNVVPQSTPIPVLTHVLIESCNDELVFTATDLTVST  43
DP3B_CHLPN|Q9Z8K0  (   5) VSRNELGNLIKKIQSVVPQNTPIPVLTHVLIETYNDELVFTATDLTVST  40
DP3B_CHLTR|O84078  (  55) ISRNELGNLIKKVQNVVPQSTPIPVLTHVLIESCNDELVFTATDLTVST  43
DP3B_HELPJ|Q9ZLX4  (   5) VSKNDLENTLRYLQAFLDKKDASSIASHIHLEVIKEKLFLKASDSDIGL  82
DP3B_LACLC|O54376  (   6) INKTAFQNALKITKQAIGSKVTIPALTKLKIEVEEKGITLIGSNGQISI  51
DP3B_MYCCA|P24117  (   5) INRIVLLDNLSKAAKVIDYKNVNPSLSGIYLNVLNDQVNVITTSGILSF  85
DP3B_MYCLE|P46387  (  17) LARESFASAVSWVAKYLPTRPTVPVLSGVLLTGSDSGLTISGFDYEVSA  39
DP3B_MYCPA|Q9L7L6  (  17) LVRESFADAVSWVAKSLPSRPAVPVLSGVLLSGTDEGLTISGFDYEVSA  30
DP3B_MYCSM|P52851  (  15) VVREDFADAVAWVARSLPTRPTIPVLAGVLLTGTDEGLTISGFDYEVSA  32
DP3B_PSEAE|Q9I7C4  (   5) IQREALLKPLQLVAGVVERRQTLPVLSNVLLVVEGQQLSLTGTDLEVEL  21
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DP3B_STRCO|P27903  (   5) VERDVLAEAVAWAARSLPARPPAPVLAGLLLKAEEGQLSLSSFDYEVSA  40
DP3B_SYNP7|P52023  (   5) CRQNELNTSLSLVSRAVPSRPNHPVLANVLLAADAGTQRLSLTAFDLSL  63
DP3B_TREPA|O83048  (   5) CEKEAFLKEISTAQEVISNKKNTSIFSNVLLAAQGALLTIRATDTKVTF  90
Q9RYE8             (   6) VTKKTLNEGLGLLERVIPSRSSNPLLTALKVETSEGGLTLSGTNLEIDL  70
Q9REN2             (   5) IINKIFTQHLKKVNRLISKNSTLPILENILITVNNGIISLTAKNLEIEL  30
Q9REN1             (   5) IKNKIFIQHLKKVNRLISKNSTLPILENILITVNNGIISLTTTNLETEL  18
Q9REN0             (   5) IKNKIFTQHLKKINRLITKNSTLPILENILITVNNGIISLTATNLETEL  18
Q9REM9             (   5) IKNKIFTQHLKKINRLISKNNTLPILENILITVNNDIVSLTATNLETEL  18
Q9PJA9             (   5) INKNTLESAVILCNAYVEKKDSSTITSHLFFHADEDKLLIKASDYEIGI 100
Q9PHE2             (   5) LQRETFLKPLAHVVNVVERRQTRSILANLLIKVNEDQLSLTGTDLEVEM  38
Q9KVX5             (   5) IERSHLIKPLQQVSGTLGGRASLPILGNLLLKVEENQLSMTATDLEVEL  35
Q9JXS8             (   6) AERDSLLKPLQAVTGIVERRHTLPILSNVLIEGKGGQTKLLATDLEIQI  37
Q9JW44             (   6) AERDSLLKPLQAVTGIVERRHTLPILSNVLIEGKGGQTKLLATDLEIQI  37
Q9EVN6             (  17) LLRESFADAVSWVAKNLPARPAVPVLSGVLLTGSDNGLTISGFDYEVSA  33
Q9EVF8             (   5) INNKIFIQNLQKINRFITKNISFPILENILISIKNDILSLTTTNLEIEL  14
Q9EVF7             (   5) INNKIFIQNLQKINRFITKNISFPILENILISIKNDILSLTTTNLEIEL  14
Q9EVF5             (   5) INNKIFIQNLQKINRFITKNISFPILENILISIKNGILSLTATNLEIEL  13
Q9EVF4             (   5) INNKIFIQNLQKINRLITKNISFPILENILISIKNGILSLTATNLEIEL  13
Q9EVF3             (   5) INNKIFIQNLQKINRFITKNISFPILENILISIKNGVLSLTATNLEIEL  17
Q9EVF2             (   5) INNKIFIHNLQKINRFVTKNISFPILENILISIKNGILSLTATNLEIEL  16
Q9EVF1             (   5) IKNSIFIKNLQKINRFVVKNISFPILENILISITNGILSLTATNLEIEL  16
Q9EVF0             (   5) IKNNIFVQHLQKVNRFITKNTSFPILENILISIKNGILSLTATNLEIEL  14
Q9EVE9             (   5) IKNNIFVQHLQNVNRFITKNTSFPILENVLISIKNRILSLTATNLEIEL  21
Q9EVE8             (   5) INNKIFIQYLKKVNRFITKNTSFPILENILISIKNGILSLTATNLEIEL  16
Q9EVE7             (   5) IKNKIFIQNLQKINRFITKNTSFPILENILISIKNGILSLTATNLEIEL  13
Q9EVE6             (   5) IKNNILIHNLQKINRFVTKNNTFPILENILISIKNGILSLTATNLEIEL  16
Q9EVE5             (   5) INNNILIKNLQKISRLLVKNTSLPILDNVLINIKNGMLSLTGTNLEIEL  20
Q9EVE4             (   5) IQNDILVENLKKITRLLIKNVSFPILENILIQIENGILSLTTTNLEIEL  23
Q9CLQ5             (   5) VSRENLLKPLQQVCGVLSSRPNIPVLNNVLLQIRGERLVITGTDLEVEL  30
Q9CJJ1             (   6) INKNAFQNALRITKQAIGSKVTIPALTKLKIEVEENGITLIGSNGQISI  45
Q9EVF6             (   5) INNKIFTQNLQKINRFITKNISFPILENILISIKDDILSLTTTNLEIEL  15
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Block IPB001001B

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DP3B_BUCAI|P57127  (  66) GTATISGRKLLDICR  15

DP3B_CAUCR|P48198  (  65) GQITAPAHTLYEIVR  45

DP3B_HAEIN|P43744  (  66) GTFTIPAKKFLDICR  16

DP3B_PSEPU|P13455  (  66) GEITVPARKLMDICK  16

DP3B_RICPR|Q9ZDB3  (  66) GEITVATQTLSDIVR  42

DP3B_SPICI|P34029  (  68) GSVLIKNKFIVEVIR  46

DP3B_STAAU|P50029  (  74) GSVVLPGRFFVDIIK  24

DP3B_STRPN|O06672  (  73) GSILLEASFFINVVS  47

DP3B_SYNY3|P72856  (  67) GRITLPAKLLNDIVS  22

DP3B_ECOLI|P00583  (  66) GATTVPARKFFDICR  12
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DP3B_PROMI|P22838  (  66) GATTVPARKFFDIWR  25
DP3B_SALTY|P26464  (  66) GATTVPARKFFDICR  12

DP3B_MYCTU|Q50790  (  77) GSVLVSGRLLSDITR  15

DP3B_AQUAE|O67725  (  65) GEVCVHSQKLYDIVK  46
DP3B_BACHD|Q9RCA1  (  73) GSIVLQAKVFAEIVK  40
DP3B_BUCAP|P29439  (  66) GKTTISGRKILNICR  17
DP3B_CHLMU|Q9PKW4  (  65) GSVTIPSRRFFQLIR  28
DP3B_CHLPN|Q9Z8K0  (  65) GAISIPSKRFFQLVK  50
DP3B_CHLTR|O84078  ( 115) GSVTIPSRRFFQLIR  28
DP3B_HELPJ|Q9ZLX4  (  66) GVGTINGKKFLDIIS  41
DP3B_LACLC|O54376  (  73) GSVLLEAAFFENVVS  36
DP3B_MYCCA|P24117  (  70) GKVLLKPKYVLEMLR 100
DP3B_MYCLE|P46387  (  77) GSVLVSGRLLSDITR  15
DP3B_MYCPA|Q9L7L6  (  77) GSVLVSGRLLSDIVR  12
DP3B_MYCSM|P52851  (  75) GSVLVSGRLLSDITK  17
DP3B_PSEAE|Q9I7C4  (  66) GEITVPARKLMDICK  16
DP3B_STRCO|P27903  (  65) GTVLVSGRLLADISR  27
DP3B_SYNP7|P52023  (  67) GAITLPAKLLNDIVS  20
DP3B_TREPA|O83048  (  65) GTTTVFCDKLVNVVS  58
Q9RYE8             (  66) ENFVVPAHLFAQIVR  64
Q9REN2             (  66) GSITVSGQKLLNICQ  23
Q9REN1             (  66) GSITVSGQKLLNICR  11
Q9REN0             (  66) GSITVSGQKLLNICR  11
Q9REM9             (  66) GSITVSGQKLLNIXQ  22
Q9PJA9             (  66) GFATANAKSIADVIK  57
Q9PHE2             (  65) GEITIPARKIYEIVR  20
Q9KVX5             (  66) GSITVPARKFLDICR   9
Q9JXS8             (  66) FRITTNAKKFQDILR  47
Q9JW44             (  66) FRITTNAKKFQDILR  47
Q9EVN6             (  77) GTVLVSGRLLSDITR  16
Q9EVF8             (  66) GNITVSSRKLLDICR   9
Q9EVF7             (  66) GNTTVSSRKLLDICR  10
Q9EVF5             (  66) GNTTVSSRKLLDICR  10
Q9EVF4             (  66) GNTTVSSRKLLDICR  10
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Q9EVF1             (  66) GSTTVSSRKLLDICR   9
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Q9EVE7             (  66) GSTTVSGRKLLDICR   9
Q9EVE6             (  66) GSTTISSRKLLDICR  10
Q9EVE5             (  66) GTATISGRKLLDICR  15
Q9EVE4             (  66) GKITISGRKILNICR  16
Q9CLQ5             (  66) GSFTIPAKKFLDICR  15
Q9CJJ1             (  73) GSVLLEAAFFENVVS  36
Q9EVF6             (  66) GNTTVSSRKLLDICR  10
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Block IPB001001C

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DP3B_BUCAI|P57127  (  99) IISGNSRYILTTLPYDSFP  22
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DP3B_CAUCR	P48198	(100)	IQAGRSRFNLPVLPAGDFP	45
DP3B_HAEIN	P43744	(99)	VQSGRSRFTLATQPAEEYP	36
DP3B_MYCTU	Q50790	(109)	LTCGNARFSLPTMPVEDYP	23
DP3B_RICPR	Q9ZDB3	(99)	IKGQNCKFNLFTLPVSSFP	70
DP3B_SPICI	P34029	(101)	IKANNFDSVLNTLNSADYP	75
DP3B_STAAU	P50029	(107)	ITSGHSEFNLSGLDPDQYP	45
DP3B_SYNY3	P72856	(106)	ITSESGRFQIRGLDADDFP	46
DP3B_ECOLI	P00583	(99)	VRSGRSRFSLSTLPAADFP	18
DP3B_PROMI	P22838	(99)	VRSGRSRFSLSTLPASDFP	21
DP3B_PSEPU	P13455	(99)	VKAGRSRFTLSTLPANDFP	20
DP3B_SALTY	P26464	(99)	VRSGRSRFSLSTLPAADFP	18
DP3B_STRPN	O06672	(106)	LTSGKSEITLKGKDSEQYP	50
DP3B_AQUAE	O67725	(97)	ITGGKSTYKLPTAPAEDFP	43
DP3B_BACHD	Q9RCA1	(106)	IRSGSSVFNLNGLDPDEYP	37
DP3B_BUCAP	P29439	(99)	ISSENSNYILSTLSADTFP	34
DP3B_CHLMU	Q9PKW4	(98)	ITSGSSCFRLLSMGKEDFP	40
DP3B_CHLPN	Q9Z8K0	(98)	ITSGSSCFRLLSMEKEDFP	47
DP3B_CHLTR	O84078	(148)	ITSGSSCFRLLSMGKEDFP	40
DP3B_HELPJ	Q9ZLX4	(98)	IKQNKSSFKLPMFDADEFP	47
DP3B_LACLC	O54376	(106)	LTSGKSEITLKGLDSEIYP	37
DP3B_MYCCA	P24117	(103)	IKTNNSDFSIGVLNSEDYP	72
DP3B_MYCLE	P46387	(109)	LTCGSARFSLPTMAVEDYP	28
DP3B_MYCPA	Q9L7L6	(109)	LNCGSARFSLPTMAVEDYP	38
DP3B_MYCSM	P52851	(107)	LTCGSARFSLPTLAVEDYP	26
DP3B_PSEAE	Q9I7C4	(99)	VKAGRSRFTLSTLPANDFP	20
DP3B_STRCO	P27903	(97)	VVCGSSRFTLHTLPVEEYP	33
DP3B_SYNP7	P52023	(100)	LSVGSGQYQMRGISADEFP	91
DP3B_TREPA	O83048	(99)	PPNKKISFQLRTLSHESFP	100
Q9RYE8		(98)	VRSGGSDFKLQTGDIEAYP	68
Q9REN2		(99)	IISDNSNYILTTLPSDNFP	16
Q9REN1		(99)	IASDNSNYILTTLPSENFP	26
Q9REN0		(99)	ISLENSNYILNTLPAENFP	31
Q9REM9		(99)	IVSDDSNYILTTLPSDNFP	33
Q9PJA9		(98)	VRQKSTKYKLPMFNHEDFP	74
Q9PHE2		(98)	LQAGRSRFTLATLPANDFP	25
Q9KVX5		(99)	VRSGRSRFSLATLPASDFP	22
Q9JXS8		(99)	LKAGKSRFALQTLPAADFP	26
Q9JW44		(99)	LKAGKSRFALQTLPAADFP	26
Q9EVN6		(109)	LTCGNARFSLPTMPVEDYP	23
Q9EVF8		(99)	IISDTSQYILRTLPADNFP	16
Q9EVF7		(99)	IISDTSQYILRTLPADNFP	16
Q9EVF5		(99)	IISNTSQYILRTLPADNFP	18
Q9EVF4		(99)	IISNTSQYILRTLPADNFP	18
Q9EVF3		(99)	IISDTSQYILRTLPADNFP	16
Q9EVF2		(99)	IISDKSQYILTTLPADNFP	15
Q9EVF1		(99)	IISDKSHYILTTLPSDNFP	16
Q9EVF0		(99)	VISDKSHYTLNTLPADNFP	18
Q9EVE9		(99)	VISEKSHYILNTLPSDDFP	20
Q9EVE8		(99)	ITSDNSHYILKTLPIDNFP	23
Q9EVE7		(99)	IISDKSHYVLTTLPADNFP	21
Q9EVE6		(99)	IISDKSKYILTTLSSNNFP	23
Q9EVE5		(99)	IISGNSRYILTTLPYDSFP	22
Q9EVE4		(99)	VSCENSNYILSTLSADDFP	24
Q9CLQ5		(99)	VKSGRSKFNLSTLPAEEYP	21
Q9CJJ1		(106)	LTSGKSEITLKGLDSEIYP	37

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Q9EVF6              (  99) IISDTSQYILRTLPADNFP  16
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Block IPB001001D

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ID   DNA_polIII_beta; BLOCK
AC   IPB001001D; distance from previous block=(15,23)
DE   DNA polymerase III, beta chain
BL   FRG;  width=24; seqs=58; 99.5%=1415; strength=1171
DP3B_BUCAI|P57127   ( 135) LKKMIEKIQFSMAKQDVRYYLNGI   8

DP3B_CAUCR|P48198   ( 136) LIRLIDKTRFAISTEETRYYLNGL  15

DP3B_MYCTU|Q50790   ( 144) FAEAISQVAIAAGRDDTLPMLTGI  14

DP3B_RICPR|Q9ZDB3   ( 135) FAKIIESTKFSISLDETRYNLNGI  20

DP3B_SPICI|P34029   ( 137) LKEIISQTSFAIGEKEKRIVFNGL  19

DP3B_STRPN|O06672   ( 142) LKKIINETAFAASTQESRPILTGV  13

DP3B_SYNY3|P72856   ( 142) LNEGLRGALFAASTDETKQVLTGV  34

DP3B_BACSU|P05649   ( 142) LKNLIRQTVFAVSTSETRPILTGV  13
DP3B_STAAU|P50029   ( 143) LKNVIAQTNFAVSTSETRPVLTGV  21

DP3B_ECOLI|P00583   ( 135) MKRLIEATQFSMAHQDVRYYLNGM  12
DP3B_HAEIN|P43744   ( 135) LRRLIEATQFSMANQDARYFLNGM  11
DP3B_PROMI|P22838   ( 135) LKRLIESTQFSMAHQDVRYYLNGM   8
DP3B_PSEPU|P13455   ( 135) LRRLIERTSFAMAQQDVRYYLNGM   8
DP3B_SALTY|P26464   ( 135) MKRLIESTQFSMAHQDVRYYLNGM  12

DP3B_AQUAE|O67725   ( 132) LVNGIEKVEYAIAKEEANIALQGM  43
DP3B_BACHD|Q9RCA1   ( 142) LKDIIRQTVFAVSTQETRPVLTGV  10
DP3B_BUCAP|P29439   ( 135) LKEMIEKTEFSMGKQDVRYYLNGM   6
DP3B_CHLMU|Q9PKW4   ( 134) LKDMFQRTSFAVSREESRYVLTGV  13
DP3B_CHLPN|Q9Z8K0   ( 134) LKTMLQRTSFAVSREESRYVLTGV  13
DP3B_CHLTR|O84078   ( 184) LKDMFQRTSFAVSREESRYVLTGV  13
DP3B_HELPJ|Q9ZLX4   ( 141) IAPVIEQTSHKRELAGVLMQFNQK 100
DP3B_LACLC|O54376   ( 142) LKEIFTETVFAVSTQENRPIFTGV  18
DP3B_MYCCA|P24117   ( 139) VKKTIYQVFVSMNENNKKLILTGL  52
DP3B_MYCLE|P46387   ( 144) FAEAIGQVAIAAGRDYTLPMLTGI  20
DP3B_MYCPA|Q9L7L6   ( 144) FAEAIGQVAIAAGRDDTLPMLTGI  14
DP3B_MYCSM|P52851   ( 142) FAEAIGQVAVAAGRDDTLPMLTGI  16
DP3B_PSEAE|Q9I7C4   ( 135) LRRLIDRTSFAMAQQDVRYYLNGM  10
DP3B_STRCO|P27903   ( 132) FASAVQQVAIAAGRDDTLPVLTGV  25
DP3B_SYNP7|P52023   ( 136) LIEGLRGTLFATSGDETKQILTGV  31
DP3B_TREPA|O83048   ( 135) LRNMINHTVFAVSEDSTRHFINGV  42
Q9RYE8              ( 133) LSRAFSSVRYAASNEAFQAVFRGI  53
Q9REN2              ( 135) LKKMIEKTQFSMGKQDVRYYLNGI   6
Q9REN1              ( 135) LKKMIEQTQFSMGKQDVRYYLNGM   5
Q9REN0              ( 135) LKKMIEKTHFSMGKQDVRYYLNGM   5
Q9REM9              ( 135) LKKMIEKTQFSMGKQDVRYYLNGI   6
Q9PJA9              ( 134) LSRSLKKILPSIDTNNPKYSLNGA  58
Q9PHE2              ( 134) LKELIERTAFAMAQQDVRYYLNGL   9
Q9KVX5              ( 135) LRGLIEKTQFSMANQDVRYYLNGM  11
Q9JXS8              ( 136) FKTMLSQVQYSMAVQDIRYYLNGL  16
Q9JW44              ( 136) FKTMLSQVQYSMAVQDIRYYLNGL  16
Q9EVN6              ( 144) FAEAISQVAIAAGRDDTLPMLTGI  14
Q9EVF8              ( 135) LKEMIEKTHFSMGKQDVRYYLNGM   5
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Q9EVF7              ( 135) LKSMIEKTHFSMGKQDVRYYLNGM   7
Q9EVF5              ( 135) LKDMIEKTHFSMGKQDVRYYLNGM   6
Q9EVF4              ( 135) LKEMIEKTHFSMGKQDVRYYLNGM   5
Q9EVF3              ( 135) IKEMIEKTHFSMGKQDVRYYLNGM   8
Q9EVF2              ( 135) LKEMIEKTHFSMGKQDVRYYLNGM   5
Q9EVF1              ( 135) LKTMIEKTHFSMGKQDVRYYLNGM   6
Q9EVF0              ( 135) LREIIEKIYFSMGKQDVRYYLNGM  11
Q9EVE9              ( 135) LKEMIEKTHFSMGKQDVRYYLNGM   5
Q9EVE8              ( 135) LRDMIEKTHFSMGKKDVRYYLNGM   9
Q9EVE7              ( 135) LKEMIEKTQFSMGKQDVRYYLNGM   5
Q9EVE6              ( 135) IREMIEKTHFSMGKQDVRYYLNGM   8
Q9EVE5              ( 135) LKKMIEKIQFSMGKQDVPIYLNGI  16
Q9EVE4              ( 135) LKEMIEKTEFSMGKQDVRYYLNGM   6
Q9CLQ5              ( 135) LRRLIEATQFSMANQDARYFLNGM  11
Q9CJJ1              ( 142) LKEIFTETVFAVSTQENRPIFTGV  18
Q9EVF6              ( 135) LKEMIEKTHFSMGKQDVRYYLNGM   5
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Block IPB001001E

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ID   DNA_polIII_beta; BLOCK
AC   IPB001001E; distance from previous block=(4,14)
DE   DNA polymerase III, beta chain
BL   TDR;  width=11; seqs=58; 99.5%=710; strength=1161
DP3B_SPICI|P34029   ( 173) ITATDSFRLSC  42

DP3B_BACSU|P05649   ( 176) CTATDSHRLAL  22
DP3B_BUCAI|P57127   ( 169) AVATDGYRLGI  12
DP3B_CAUCR|P48198   ( 174) AVATDGHRLAL  13
DP3B_ECOLI|P00583   ( 169) TVATDGHRLAV  12
DP3B_HAEIN|P43744   ( 169) TVATDGHRLAV  12
DP3B_MYCTU|Q50790   ( 178) LAATDRFRLAV  21
DP3B_PROMI|P22838   ( 169) TVATDGHRLAV  12
DP3B_PSEPU|P13455   ( 169) AVSTDGHRLAL  19
DP3B_RICPR|Q9ZDB3   ( 169) AASTDGYRLSI  24
DP3B_SALTY|P26464   ( 169) TVATDGHRLAV  12
DP3B_STAAU|P50029   ( 177) CTATDSHRLAV  21
DP3B_STRPN|O06672   ( 177) TVATDSHRLSQ  21
DP3B_SYNY3|P72856   ( 176) FAATDGHRLAV  15

DP3B_AQUAE|O67725   ( 166) FVGSDGHRLAL  47
DP3B_BACHD|Q9RCA1   ( 176) CTATDSHRLAM  23
DP3B_BUCAP|P29439   ( 169) SVATDGYRLAI  13
DP3B_CHLMU|Q9PKW4   ( 168) VVGTDGKRLAK  25
DP3B_CHLPN|Q9Z8K0   ( 168) IVGTDGKRLAK  26
DP3B_CHLTR|O84078   ( 218) VVGTDGKRLAK  25
DP3B_HELPJ|Q9ZLX4   ( 170) VVGTDTKRLSY  41
DP3B_LACLC|O54376   ( 177) AVATDSHRMSQ  28
DP3B_MYCCA|P24117   ( 173) FSTTDSFRISQ 100
DP3B_MYCLE|P46387   ( 178) LAATDRFRLAV  21
DP3B_MYCPA|Q9L7L6   ( 178) LAATDRFRLAV  21
DP3B_MYCSM|P52851   ( 176) LAATDRFRLAV  21
DP3B_PSEAE|Q9I7C4   ( 169) SVATDGHRLAM  15
DP3B_STRCO|P27903   ( 166) LASTDRYRFAV  48
DP3B_SYNP7|P52023   ( 170) FAATDGHRLAV  15
DP3B_TREPA|O83048   ( 169) CVSTDGKRLAY  25
Q9RYE8              ( 167) VVASDGYRVAI  65
Q9REN2              ( 169) AVATDGYRLGI  12
Q9REN1              ( 169) AVATDGYRLGI  12
Q9REN0              ( 169) TVATDGYRLGI  13
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Q9REM9              ( 169) AVATDGYRLGI  12
Q9PJA9              ( 168) FVGTDTKRLAI  34
Q9PHE2              ( 168) CVATDGHRLAL  14
Q9KVX5              ( 169) SVATDGHRMAV  21
Q9JXS8              ( 170) LVATDGHRLAY  14
Q9JW44              ( 170) LVATDGHRLAY  14
Q9EVN6              ( 178) LAATDRFRLAV  21
Q9EVF8              ( 169) MVATDGYRLGM  14
Q9EVF7              ( 169) MVATAGYRLGM  72
Q9EVF5              ( 169) MVATDGYRLGI  12
Q9EVF4              ( 169) MVATDGYRLSI  14
Q9EVF3              ( 169) MVATDGYRLGI  12
Q9EVF2              ( 169) MVATDGYRLGI  12
Q9EVF1              ( 169) MVATDGYRLGI  12
Q9EVF0              ( 169) MVATDGYRLGI  12
Q9EVE9              ( 169) IVATDGYRLGT  28
Q9EVE8              ( 169) MVATDGYRLAL  12
Q9EVE7              ( 169) MVATDGYRLAI  11
Q9EVE6              ( 169) MIATDGYRLGI  35
Q9EVE5              ( 169) AVATDGYRLGI  12
Q9EVE4              ( 169) SVATDGYRLAI  13
Q9CLQ5              ( 169) TVATDGHRLAV  12
Q9CJJ1              ( 177) AVATDSHRMSQ  28
Q9EVF6              ( 169) MVATDGYRLGM  14
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Block IPB001001F

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ID   DNA_polIII_beta; BLOCK
AC   IPB001001F; distance from previous block=(9,25)
DE   DNA polymerase III, beta chain
BL   VPE;  width=11; seqs=58; 99.5%=720; strength=1034
DP3B_RICPR|Q9ZDB3   ( 193) VILPQKSAEEI  51

DP3B_SPICI|P34029   ( 196) VIIPGKFINEI  42

DP3B_STRPN|O06672   ( 201) VVIPSRSLREF  33

DP3B_SYNY3|P72856   ( 212) VTIPARALREL  31

DP3B_BACSU|P05649   ( 201) VVIPGKSLTEL  21
DP3B_STAAU|P50029   ( 201) VIIPGKALAEL  17

DP3B_BUCAI|P57127   ( 193) IVIPRKGVIEL  13
DP3B_CAUCR|P48198   ( 199) VIVPRKTIAEA  17
DP3B_ECOLI|P00583   ( 193) VIVPRKGVIEL  11
DP3B_HAEIN|P43744   ( 193) VILPRKGVLEL  14
DP3B_MYCTU|Q50790   ( 204) VLVPAKTLAEA  23
DP3B_PROMI|P22838   ( 193) VIVPRKGVIEL  11
DP3B_PSEPU|P13455   ( 194) VIVPRKGILEL  11
DP3B_SALTY|P26464   ( 193) VIVPRKGVIEL  11

DP3B_AQUAE|O67725   ( 187) LLIPRKSLKVL  66
DP3B_BACHD|Q9RCA1   ( 203) VVIPGKSLNEL  21
DP3B_BUCAP|P29439   ( 193) IIIPNKAVMEL  29
DP3B_CHLMU|Q9PKW4   ( 193) YIIPIKAVEEI  28
DP3B_CHLPN|Q9Z8K0   ( 193) YIIPIKAVEEI  28
DP3B_CHLTR|O84078   ( 243) YIIPIKAVEEI  28
DP3B_HELPJ|Q9ZLX4   ( 197) CILPKRALLEI  51
DP3B_LACLC|O54376   ( 201) VILPSKSINSF  40
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DP3B_MYCCA|P24117   ( 198) ITIPFKTALEL  53
DP3B_MYCLE|P46387   ( 204) VLVPAKTLVEV  39
DP3B_MYCPA|Q9L7L6   ( 204) VLVPAKTLAEA  23
DP3B_MYCSM|P52851   ( 202) VLVPAKTLAEA  23
DP3B_PSEAE|Q9I7C4   ( 194) VIVPRKGILEL  11
DP3B_STRCO|P27903   ( 192) ALVPAKTLQDT  70
DP3B_SYNP7|P52023   ( 196) VTVPSRALRDL  48
DP3B_TREPA|O83048   ( 194) VIVPTKILGIV 100
Q9RYE8              ( 189) LIIPARSVDEL  40
Q9REN2              ( 193) AIIPRRGIIEL  21
Q9REN1              ( 193) VIIPRKGIIEL  10
Q9REN0              ( 193) AIIPRKGIIEL  16
Q9REM9              ( 193) VIIPRRGIIEL  15
Q9PJA9              ( 191) FSIPKKAIMEM  91
Q9PHE2              ( 193) IILPRKGVMEL  16
Q9KVX5              ( 193) IIVPRKGVLEL  12
Q9JXS8              ( 194) VILPRKTVLEL  15
Q9JW44              ( 194) VILPRKTVLEL  15
Q9EVN6              ( 204) VLVPAKTLAEA  23
Q9EVF8              ( 195) IIMTRKGIIEL  18
Q9EVF7              ( 195) IIMTRKGIIEL  18
Q9EVF5              ( 194) IVMTRKGIIEL  21
Q9EVF4              ( 194) IIMTRKGIIEL  18
Q9EVF3              ( 194) IIMTRKGIIEL  18
Q9EVF2              ( 194) IVMTRRGIIEL  26
Q9EVF1              ( 194) IIIARKGIIEL  21
Q9EVF0              ( 195) IIIARKGITEL  23
Q9EVE9              ( 195) IIIARKGITEL  23
Q9EVE8              ( 194) IVIARQGIIEL  47
Q9EVE7              ( 195) IVITRQGIIEL  41
Q9EVE6              ( 194) IIITRKGITEL  17
Q9EVE5              ( 193) IVIPRKGVIEL  13
Q9EVE4              ( 193) IIIPSKAVMEL  17
Q9CLQ5              ( 193) VILPRKGVLEL  14
Q9CJJ1              ( 201) VILPSKSINSF  40
Q9EVF6              ( 195) IIMTRKGIIEL  18
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Block IPB001001G

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ID   DNA_polIII_beta; BLOCK
AC   IPB001001G; distance from previous block=(21,36)
DE   DNA polymerase III, beta chain
BL   SGP;  width=16; seqs=58; 99.5%=1004; strength=1179
DP3B_BUCAI|P57127   ( 234) TQLIEGQYPDYKSVLL  18

DP3B_MYCTU|Q50790   ( 251) TRLLDAEFPKFRQLLP  30

DP3B_RICPR|Q9ZDB3   ( 238) SKLIDGTFPDYSAFIP  50

DP3B_SPICI|P34029   ( 228) QKIIEGKYPDTSKVIR  83

DP3B_SYNY3|P72856   ( 255) SRKLEGAYPAYDQLIP  68

DP3B_BACSU|P05649   ( 242) SRLLDGNYPDTTSLIP  27
DP3B_STAAU|P50029   ( 242) SRLLEGHYPDTTRLFP  34
DP3B_STRPN|O06672   ( 242) TRLLEGNYPDTDRLIP  25

DP3B_CAUCR|P48198   ( 240) SKVIDGAFPDYMRVIP  32
DP3B_ECOLI|P00583   ( 234) SKLVDGRFPDYRRVLP  21
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DP3B_HAEIN|P43744   ( 234) SKLIDGRFPDYRRVLP  16
DP3B_PROMI|P22838   ( 235) SKLVDGRFPDYRRVLP  21
DP3B_PSEPU|P13455   ( 235) SKLVDGKFPDYERVLP  20
DP3B_SALTY|P26464   ( 234) SKLVDGRFPDYRRVLP  21

DP3B_AQUAE|O67725   ( 229) VRLLEGEFPDYMSVIP  24
DP3B_BACHD|Q9RCA1   ( 244) SRLLEGKYPVTKNMIP  72
DP3B_BUCAP|P29439   ( 234) TQLIEGEYPDYKSVLF  22
DP3B_CHLMU|Q9PKW4   ( 233) TKLLSGEFPDFSPVIS  36
DP3B_CHLPN|Q9Z8K0   ( 233) TKLLSGEFPDFSPVIS  36
DP3B_CHLTR|O84078   ( 283) TKLLSGEFPDFSPVIS  36
DP3B_HELPJ|Q9ZLX4   ( 235) TKLIDGNYPDYQKILP  29
DP3B_LACLC|O54376   ( 242) SRLIEGSYPDTNRLIP  24
DP3B_MYCCA|P24117   ( 238) SNLIDGKFPNVQIAFP 100
DP3B_MYCLE|P46387   ( 251) TRLLDAEFPKFRQLLP  30
DP3B_MYCPA|Q9L7L6   ( 251) TRLLDAEFPKFRQLLP  30
DP3B_MYCSM|P52851   ( 249) TRLLDAEFPKFRQLLP  30
DP3B_PSEAE|Q9I7C4   ( 235) SKLVDGKFPDYERVLP  20
DP3B_STRCO|P27903   ( 237) TRLLEGDLPKYKTLFP  73
DP3B_SYNP7|P52023   ( 239) SRTLDGQYPNYGQLIP  54
DP3B_TREPA|O83048   ( 234) SVLIEGQFPNYKRVIP  39
Q9RYE8              ( 228) LKLLDGDFPDYERVIP  39
Q9REN2              ( 234) VQLIEGEYPDYKSVLL  19
Q9REN1              ( 234) VQLIEGEYPDYKSILL  21
Q9REN0              ( 234) VQLIEGEYPDYKSILS  25
Q9REM9              ( 234) VQLIEGEYPDYKSILL  21
Q9PJA9              ( 229) TKLINDKFPDYEKVIP  83
Q9PHE2              ( 234) SKLIDGSFPDYEGVIP  23
Q9KVX5              ( 234) SKLVDGRFPDYRRVLP  21
Q9JXS8              ( 235) SKVIDGKFPDFNRVIP  25
Q9JW44              ( 235) SKVIDGKFPDFNRVIP  25
Q9EVN6              ( 251) TRLLDAEFPKFRQLLP  30
Q9EVF8              ( 236) AQLIEGKFPNYDSLFL  18
Q9EVF7              ( 236) AQLIEGKFPNYDSLFL  18
Q9EVF5              ( 235) AQLIEGKFPNYNSIFL  20
Q9EVF4              ( 235) AQLIEGIFPNYDSLFL  28
Q9EVF3              ( 235) AQLIEGKFPNYDSVFL  18
Q9EVF2              ( 235) AQLIEGKFPNYDSLFL  18
Q9EVF1              ( 235) AQLLEGKFPNYDSLFV  37
Q9EVF0              ( 236) AQLIEGKFPDYESIFL  18
Q9EVE9              ( 236) AQLIEGKFPNYESIFL  19
Q9EVE8              ( 235) AQLIEGTFPNYTSVFL  25
Q9EVE7              ( 236) AQLIEGKFPNYESIFL  19
Q9EVE6              ( 235) AQLIEGQFPNYESVLF  23
Q9EVE5              ( 234) TQLIEGQYPDYKSVLL  18
Q9EVE4              ( 234) TQLIEGEYPDYESVLF  22
Q9CLQ5              ( 234) SKLIDGRFPDYRRVLP  16
Q9CJJ1              ( 242) SRLIEGSYPDTNRLIP  24
Q9EVF6              ( 236) AQLIEGKFPNYNGLFL  24
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Block IPB001001H

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ID   DNA_polIII_beta; BLOCK
AC   IPB001001H; distance from previous block=(58,71)
DE   DNA polymerase III, beta chain
BL   GYL;  width=20; seqs=58; 99.5%=1114; strength=1193
DP3B_BACSU|P05649   ( 323) GEELNISFSPKYMLDALKVL  40

DP3B_BUCAI|P57127   ( 312) GNTVKISINVYYIIEILNSI  19
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DP3B_CAUCR	P48198	(318)	GEPFEIGFNARYLLDVCGQI	47
DP3B_HAEIN	P43744	(312)	GEELEVGFNVTYILDVLNAL	15
DP3B_MYCTU	Q50790	(328)	GEPLTIAFNPTYLTDGLSSL	15
DP3B_PSEPU	P13455	(313)	GSSLEIGFNVSYLLDVLGVM	27
DP3B_RICPR	Q9ZDB3	(325)	DESLVIGFNPQYLEDVLKAI	35
DP3B_SPICI	P34029	(308)	GTDQIIAFNSKYILDALKAF	37
DP3B_STAAU	P50029	(322)	GGSLKISFNSKYMMDALKAI	21
DP3B_STRPN	O06672	(322)	GEDLTISFNPTYLIDSLKAL	21
DP3B_SYNY3	P72856	(335)	GEGGQIAFNIKYLMDGLKAL	32
DP3B_ECOLI	P00583	(312)	GAEMEIGFNVSYVLDVLNAL	21
DP3B_PROMI	P22838	(313)	GEEMEIGFNVSYLLDVLNTL	13
DP3B_SALTY	P26464	(312)	GTEMEIGFNVSYVLDVLNAL	17
DP3B_AQUAE	O67725	(307)	GEPFEIGFNGKYLMEALDAY	39
DP3B_BACHD	Q9RCA1	(325)	GEELRISFNGKNVIDALKVV	43
DP3B_BUCAP	P29439	(312)	GEKIEISINVYYLLDVINNI	23
DP3B_CHLMU	Q9PKW4	(311)	GETLEIAFNPFFFLDILKHS	23
DP3B_CHLPN	Q9Z8K0	(311)	GELLEIAFNPFFFLDILKHS	28
DP3B_CHLTR	O84078	(361)	GETLEIAFNPFFFLDILKHS	23
DP3B_HELPJ	Q9ZLX4	(313)	EKAFHLGVNAKFFLEALNAL	64
DP3B_LACLC	O54376	(324)	GNDLSISFNPEYLIDALKVI	16
DP3B_MYCCA	P24117	(319)	NKSLSISFNTRFLIDAIKTL	41
DP3B_MYCLE	P46387	(328)	GEPLTIAFNPNYLTDGLASV	27
DP3B_MYCPA	Q9L7L6	(328)	GEPLTIAFNPTYLTDGLGSV	18
DP3B_MYCSM	P52851	(326)	GEPLTIAFNPTYLTDGLGSL	15
DP3B_PSEAE	Q9I7C4	(313)	GGNLEIGFNVSYLLDVLGVI	15
DP3B_STRCO	P27903	(314)	GDDISIAFNPTFLLDGLSAI	17
DP3B_SYNP7	P52023	(319)	GEPLEIAFNVRYLAEGLKAL	26
DP3B_TREPA	O83048	(316)	GESEVIALNYLYLEEPLKVF	74
Q9RYE8		(308)	EQAMSLAFNARHVLDALGPI	59
Q9REN2		(312)	GDDIEISINVYYIIEVLNVI	13
Q9REN1		(312)	GDDIEISINVYYIIEVLSVI	15
Q9REN0		(312)	GENIEISINVYYIIEVLNVI	15
Q9REM9		(312)	GDDIEISINVYYIIEVLNVI	13
Q9PJA9		(304)	SEEFNLTIKIKHLLDFLTSI	100
Q9PHE2		(312)	VDGLAIGFNVNYLLDALSSL	32
Q9KVX5		(312)	GEPIEIGFNVSYILDVLNTL	12
Q9JXS8		(313)	GGELEVGFNIGYLMDVLRNI	28
Q9JW44		(313)	GGELEVGFNIGYLMDVLRNI	28
Q9EVN6		(328)	GEPLTIAFNPTYLTDGLSSL	15
Q9EVF8		(314)	GPSIKISINVYYILDILNSI	9
Q9EVF7		(314)	GPSIKISINVYYILDILNSI	9
Q9EVF5		(313)	GPSIKISINVYYILDVLNSI	9
Q9EVF4		(313)	GPSIKISINVYYILDVLNSI	9
Q9EVF3		(313)	GPSIKISINVYYMLDILNSI	13
Q9EVF2		(313)	GPSIKISINVYYILDILNSI	9
Q9EVF1		(313)	GPSIKISINVYYILDILNAI	9
Q9EVF0		(314)	GPSIEISINVYYILDILNVI	9
Q9EVE9		(314)	GPSIEISINVYYILDILNVI	9
Q9EVE8		(313)	NPSIEISINVYYILDILNTI	16
Q9EVE7		(314)	GPEIEISINVYYILDILNAI	9
Q9EVE6		(313)	GPVIEISINVYYILDILNSI	15
Q9EVE5		(312)	GNTVKISINVYYIIEILNSI	19
Q9EVE4		(312)	GDTIEISINVYYLLDVINNI	19
Q9CLQ5		(312)	GEEMEVGFNVSYILDVLNAL	17

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Q9CJJ1          ( 324) GNDLAISFNPEYLIDALKVI  18
Q9EVF6          ( 314) GPSIKISINVYYILDILNSI   9
//
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COBBLER sequence (region containing Blocks only)

To do a BLAST search, copy the cobbler sequence below then click on a BLAST link

[Blast Search] [Gap-Blast Search] [PSI-Blast Search]

COBBLER sequence:
>IPB001001 DP3B_PROMI|P22838 from 1 to 342 with embedded consensus blocks
mkfiINRNTLLESLQKVSRVISKRTTIPILSNILIEVENDQLTLTGTDLEISLmarvslsqsheiGSVTVPARKFLDIVR
glpegaeisveldgdrllITSGNSRFTLRTLPAEDFPnlddwqseveftlpqatLKEMIEQTQFAMSKQDTRYYLNGVlf
etentelrMVATDGYRLAVcamdigqslpghsVIIPRKGIMELmrlldgsgesllqlqigsnnlrahvgdfiftSKLIDG
KFPDYERVIPknptktviagcdilkqafsraailsnekfrgvrinltngqlkitannpeqeeaeeivdvqyqGEELEISF
NVKYLLDVLNAIkceevkllllt

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Additional Links (separate browser window)

MetaFam IPB001001

IPB001001A : CYRCA IPB001001A

[Blocks home]

WEST

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L1: Entry 12 of 16 File: USPT Mar 18, 1997

DOCUMENT-IDENTIFIER: US 5612182 A
TITLE: Mycobacteriophage specific for the mycobacterium tuberculosis complex

Detailed Description Text (29):
A second potential open reading frame ORF (ORF 1 of NheI-D) was aligned with the DNA polymerase III .beta.-subunit of Streptomyces coelicolor. The polymerase III .beta. subunit is the product of the S. coelicolor dnaN gene. The alignment showed significant homology of 35% over 360 amino acids. It is likely that translation of ORF 1 (NheI-D) begins at the valine GTG initiator at nucleotide 390. Use of these sequences for translation allows good alignment of both the amino and C-terminal portions of the proteins. ORF 1 (NheI-D) also shows weaker hornology to the analogous proteins from E. coli and B. subtilis, probably as a result of the closer phylogenetic relationship between mycobacteria and streptomyces than between mycobacteria and E. coli or B. subtilis. However, class III-type DNA polymerases were previously unknown in phage. Phage polymerases are either of type I (Taq, klenow, L5 phage, T coliphages) or of type II (phi29). The type III enzymes are multisubunit enzymes previously found only in bacteria where they are known to be involved in DNA replication and repair. The beta subunit is not known to catalyze DNA replication by itself, but instead appears to play a role as a DNA clamp which provides processivity. Thus, if ORF 1 (NheI-D) is a bona fide DNA polymerase subunit, the other subunits might reside in the DS6A genome, or be supplied by the host cell. The highly processive nature of class III DNA polymerases makes them desirable for use in vitro in nucleic acid amplification and DNA syntheses, etc. ORF 1 (NheI-D) of DS6A may therefore be cloned and expressed in transformed host cells to produce a new recombinant class III DNA polymerase useful in these methods.

L1: Entry 10 of 16 File: USPT May 27, 1997

DOCUMENT-IDENTIFIER: US 5633159 A
TITLE: DNA polymerase III .beta.-subunit from mycobacteriophage DS6A

Detailed Description Text (29):
A second potential open reading frame ORF (ORF 1 of NheI-D) was aligned with the DNA polymerase III .beta.-subunit of Streptomyces coelicolor. The polymerase III .beta. subunit is the product of the S coelicolor dnaN gene. The alignment showed significant homology of 35% over 360 amino acids. It is likely that translation of ORF 1 (NheI-D) begins at the valine GTG initiator at nucleotide 390. Use of these sequences for translation allows good alignment of both the amino and C-terminal portions of the proteins. ORF 1 (NheI-D) also shows weaker homology to the analogous proteins from E. coli and B. subtilis, probably as a result of the closer phylogenetic relationship between mycobacteria and streptomyces than between mycobacteria and E. coli or B. subtills. However, class III-type DNA polymerases were previously unknown in phage. Phage polymerases are either of type I (Taq, klenow, L5 phage, T coliphages) or of type II (phi29). The type III enzymes are multisubunit enzymes previously found only in bacteria where they are known to be involved in DNA replication and repair. The beta subunit is not known to catalyze DNA replication by itself, but instead appears to play a role as a DNA clamp which provides processivity. Thus, if ORF 1 (NheI-D) is a bona fide DNA polymerase subunit, the other subunits might reside in the DS6A genome, or be supplied by the host cell. The highly processive nature of class III DNA polymerases makes them desirable for use in vitro in nucleic acid amplification and DNA syntheses, etc. ORF 1 (NheI-D) of DS6A may therefore be cloned and expressed in transformed host cells to produce a new recombinant class III DNA polymerase .beta.-sub unit useful in these methods.